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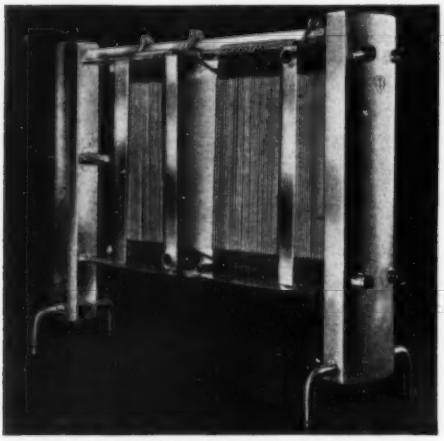

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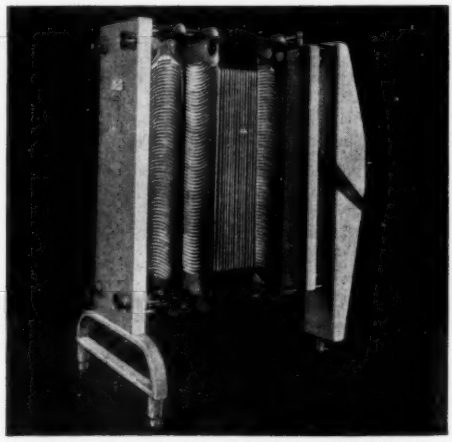

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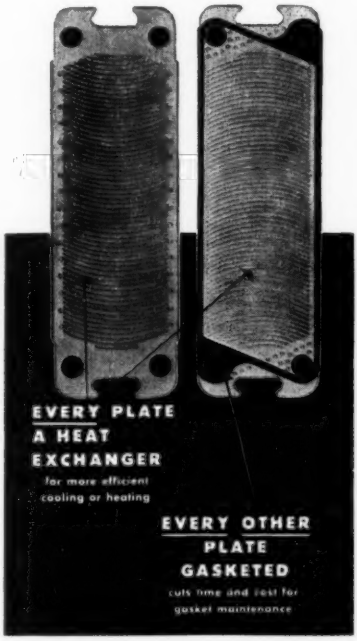

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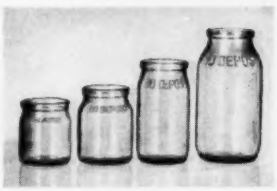

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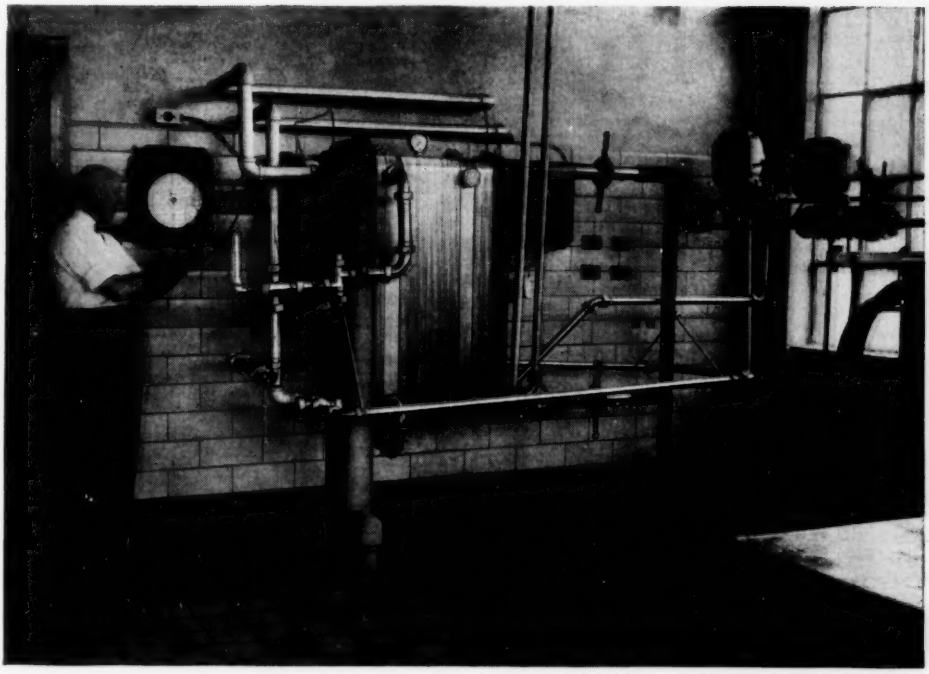

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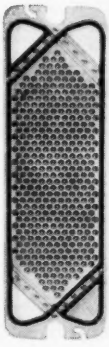

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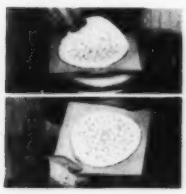

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVIII JUNE, 1955 NUMBER 6

ABSTRACTS OF PAPERS PRESENTED AT THE FIFTIETH ANNUAL MEETING

Michigan State College
East Lansing
June 20-23, 1955

MANUFACTURING SECTION

M1. The activity of lactic acid starters in reconstituted nonfat dry milk solids and fluid milks. V. W. GREENE and J. J. JEZESKI, Univ. of Minnesota, St. Paul.

A sensitive and practical technique which relies on speed of acid production as well as reductive capacity has been developed to evaluate starter activity. This test was employed to compare the activity of starters in lots of reconstituted nonfat dry milk which had different predrying heat histories. A survey of 64 commercial NFDMS samples from all parts of the country showed that there was a definite correlation between undenatured serum protein content and starter activity. Samples which had evidently undergone an extremely high heat treatment and most "low heat" samples were inferior to those which contained between 2 and 5 mg/g of undenatured serum protein. Further studies on fluid milks again illustrated the dependence of starter activity on heat treatment. It was also shown that the quality of the original raw milk and the method of handling, as well as the individual characteristics of the starter employed, all interacted to influence starter development. Possible explanations for the observed phenomena are presented.

M2. Preparation of starter cultures by combining separate strains of a lactic streptococcus and an associate at the final propagation. D. D. DEANE and F. E. NELSON, Iowa State College, Ames.

This study was conducted to investigate the possibility of preparing starter cultures that would produce adequate amounts of acetylmethylcarbinol (AMC) and biacetyl (AC_2) by waiting until the final propagation before combining a single strain of *Streptococcus lactis* or *S. cremoris* and a suitable *Leuconostoc* associate.

The lactic streptococci were isolated from raw milk and cream and commercial starters. The *Leuconostoc* strains came mainly from raw cream, although a few commercial cultures also were plated. The lactic streptococci from commercial cultures were the most active when first isolated; however, almost all those isolated became less active after repeated transfers, and only a few continued to produce sufficient lactic acid to be combined with a *Leuconostoc* culture.

The best results were obtained when 1% of *S. lactis* or *S. cremoris* and 0.2% of a suitable *Leuconostoc* culture were used with an incubation period of 18 hr. at 21° C. Both reconstituted skimmilk and homogenized whole milk standardized to 2% milk fat were found to be satisfactory media. The amounts of AMC + AC_2 produced by the most effective combinations studied equaled those produced by some commercial cultures. Most combinations, however, produced less and many produced little, if any, of these compounds.

M3. Viscosity of reconstituted nonfat dried milks. W. D. RUTZ and C. H. WHITNAH, Kansas State College, Manhattan.

Viscosities of reconstituted nonfat dried milks were determined by the Ostwald pipette technique. Samples studied included high and low heat powders made from the same skimmilk and three brands of commercial "instant soluble" nonfat dry milk. The viscosity of these skimmilks was compared with that of fluid skimmilk.

After reconstituting the dried milks in water at about 4° C. they were stored at that temperature until viscosities were measured. The measurements were at 4° C. at intervals ranging from 0.25 to 370 hr.

Initial viscosities of both fluid and reconstituted nonfat dry milks ranged from 2.7 to 3.6 cps. With some of the reconstituted powders no consistent increase in viscosity during aging was observed. The rate of increase in viscosity

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of fresh skimmilk during aging was more than twice as great as for any reconstituted powder.

When head pressure of the viscometer was reduced by inclining the instrument and the length of flow times was tripled, viscosities of fresh skimmilk varied less than 1% from trials in which the viscometer was vertical. The differences in viscosities of reconstituted powders when determined in both vertical and inclined viscometers varied from 2% in a low heat powder to 20% in one of the commercial powders.

M4. Studies on sterile milk. E. O. Herreid and Lalitha Kadaba, Univ. of Illinois, Urbana.

In pilot plant operations milk was sterilized in a Mallory heater at different temperatures, aseptically bottled, and stored at 40°, 70°, and 100° F. It was examined organoleptically for ascorbic acid, carotene, vitamin A, thiamine, phosphatase, total N, casein N, noncasein N, nonprotein N, ammonia N, and for fat stability.

The cooked flavor in the sterile milk gradually disappeared in about 72 to 96 hr. At 40° F. the palatable flavor was maintained for 5 to 6 mo.; at 70° F., for about 2½ to 3 mo.; and at 100° F. for about 2 to 3 wk. In milk sterilized at 300° F. ascorbic acid decreased about 15%; at 40° F. it had decreased about 40%; at 70° F., about 60%; and at 100° F., about 80%. There were no significant changes in carotene and vitamin A. Thiamine decreased about 10% during sterilization and showed an apparent increase during storage at 40° and 70° F., due probably to other interfering substances, but it was always lowest at 100° F. Phosphatase reactivation was greatest at 70° F., next at 100° F., and least at 40° F. Small increases in nonprotein N were found. Thiobarbituric acid and peroxide values were always highest in the milks stored at 100° F. and lowest at 40° F.

M5. Effect of HTST heat treatment of separated milk on the quality of spray-dried nonfat dry milk solids for manufacture of cottage cheese. W. K. Stone, E. J. Finnegan, and G. C. Graf, Virginia Polytechnic Inst., Blacksburg.

Fresh skimmilk was preheated for various time-temperature combinations and condensed. The condensed milk (36%) was forewarmed to 140° F., homogenized at 2,000 lb. pressure, and dried. Part of the powder was removed during drying and part after drying. It was packaged in 100-lb. polyethylene-lined paper bags. Solubility index, moisture, scorched particles, and whey protein nitrogen were determined on each dried product.

Cottage cheese was "set up" in 11.0% skimmilk reconstituted from each condensed product and from each powder. Curd tension measurements during manufacture and firmness of curd after draining were used to evaluate curd-forming properties. Titratable acidity and pH were made at various intervals.

High preheat treatments produced powders with poor curd-forming properties. Powders from skimmilk preheated to 163° F. for 16 sec. plus 185° F. for 10 min. demonstrated low curd tension and formed a mushy consistency at cutting. Those produced from skimmilk preheated to 163° F. for 16 sec. demonstrated high curd tension and formed a firm curd at cutting. A pH of 4.8-4.7 appeared optimum at cutting.

M6. The manufacture of Ricotta cheese from whole milk. H. M. Windlan and F. V. Kosikowski, Cornell Univ., Ithaca, N. Y.

Significant increases in the production of a soft Italian type cheese known as Ricotta prompted a detailed study into the nature and mode of manufacturing this cheese. In the past, Ricotta cheese has been manufactured solely from whey and small portions of skimmilk. The demand for a softer, creamier product has resulted in a shift of emphasis so that a great deal of Ricotta is manufactured from whole milk. Technical problems encountered deal not only with conditions necessary for heat precipitation of whey proteins but also with the casein present in large concentrations. Two types of precipitations occur in the manufacture of whole milk Ricotta and, if improperly produced, may result in inferior cheese and heavy losses in yield. Other unique problems exist in this industry.

To manufacture this Ricotta cheese, fresh whole milk is acidified by the addition, before heating, of appropriate amounts of lactic acid starter. Heat is applied by a combination of direct steam injection and by steam under pressure into the jacket of the kettle. The resulting cheese is very soft in texture with a pleasant cooked flavor. To maintain quality, the product must be refrigerated.

M7. A method for determining time for cutting curd for cottage cheese. D. B. Emmons, W. V. Price, and A. M. Swanson, Univ. of Wisconsin, Madison.

Measurements of titratable acidity and pH are ineffective for determining time for cutting curd made from reconstituted skimmilk and from excessively heated skimmilk. An acid coagulation (A-C) test was developed to select the optimum time for cutting to obtain the best curd-handling characteristics in making cottage cheese by the short-set process.

After adding starter, and just before adding rennet to the vat, a portion of skimmilk is removed in a beaker for the A-C test sample. The rennet is then added to the vat and the beaker is supported in the vat so that the two milk levels are the same. When a gel is formed in the test sample, it is carefully cut once or twice with a spatula. Cutting is repeated at intervals of approximately 5 min. until lines of whey first appear where the knife cut the gel. This indicates the optimum time for cutting the curd in the vat. It is termed the A-C end point.

The A-C test is simple and reproducible, makes pH and titratable acidity measurements unnecessary, directly detects optimum properties of coagulum required for curd-handling operations, and is particularly useful in manufacturing cottage cheese from reconstituted skimmilk and from excessively heated skimmilk.

M8. Cheddar cheese made from pasteurized milk homogenized at various pressures. I. I. PETERS, Texas Agr. Expt. Sta., College Station.

Cheddar cheese was made by the conventional method with either Holstein or Jersey milk. Jersey milk was standardized with natural skimmilk to a fat:casein ratio of approximately 1:0.7; Holstein milk was used unstandardized.

Each lot of milk was pasteurized at 143° F. for 30 min. and cooled to 120° F., after which portions were withdrawn and homogenized at pressures of 0, 500, 1,000, and 2,000 p.s.i., then cooled to 40° F. over a surface cooler. The milk was held over night and made into cheese the next morning.

In general, increasing homogenization pressures resulted in decreasing elasticity of curd, slower expulsion of whey from curd, decreasing fat losses in whey, and a decline in color intensity of cheese. Increasing homogenization pressures applied to milk progressively reduced fat leakage in cheese to a minimum of 50% or less of control cheese made from milk homogenized at 0 p.s.i.

Ranking of 6-mo.-old cheese, based on flavor, body, and texture, placed the Holstein milk cheese in the following order from top to bottom: 500, 1,000, 0, 2,000; and the Jersey milk cheese: 1,000, 500, 0, 2,000. Duplicate trials gave similar results.

M9. Studies in the ripening of surface ripened cheese. S. L. TUCKEY and M. R. SAHASRABUDHE, Univ. of Illinois, Urbana.

Chromatographic analysis for amino acids in Limburger cheese with Whatman No. 1 filter paper demonstarted that ripening proceeded more rapidly in the outer surface area than in the interior, as measured by number of amino acids liberated, as well as quantity.

Each agent (mixed lactic starter, *B. linens*, a yeast culture, and rennet extract, singly and in combination) was studied to determine its relative contribution to the ripening process. All agents demonstrated proteolytic activity in sterile milk at 55-60° F. However, the yeast culture exhibited only slight proteolysis.

Data showing changes in the carbohydrates and the fatty and organic acids, as well as in amino acids, will be presented.

M10. Effect of fatty acids in pasteurized Liederkranz cheese on the growth of Clostridium botulinum added to the product. N. GRECZ, R. O. WAGENAAR, and G. M. DACK, Food Research Institute, Univ. of Chicago.

Three groups of experiments, each including 5 trials at 2-wk. intervals, were conducted with portions of the same batch of Liederkranz cheese stored at 2-4° C. The cheese was approximately 4 wk. old at the initial analysis. Quantitative fatty acid analyses were made with the chromatographic method of Harper (*J. Dairy Sci.*, **36**: 808-16. 1953) on a sample from each cheese preparation. These preparations were pasteurized at 90° C. for 10 min., cooled, and subsequently inoculated with toxin-free spores of *Cl. botulinum*. After incubation under anaerobic conditions at 30° C. they were analyzed at intervals for toxin.

The analyses showed that Liederkranz cheese must be given more than 1 mo. additional aging at 2-4° C. before it becomes a poorer medium for growth and toxin production of *Cl. botulinum*.

The data on the development of fatty acids during aging at 2-4° C. showed that acetic acid remained relatively constant throughout each experiment. On aging of the cheese, butyric acid increased at a faster rate than propionic. All the acids of butterfat with 5 or more carbons were titrated as a group. Their level increased steadily during aging.

M11. Preliminary studies of the Australian short-time method of making Cheddar cheese. P. A. DOWNS, Univ. of Nebraska, Lincoln.

The Australian modification of the USDA method for making American Cheddar cheese by the use of *St. durans* has been carried out on the commercial as well as experimental basis. The method when applied to raw milk resulted in an open cheese because of Swiss eye formation. This was noted especially when cheese was cured at 60-65° F.

The method applied to pasteurized milk (165° F. with minimum hold period) resulted in cheese which was commercially acceptable. The commercial production with 3,000-lb. vats of milk resulted in cheese which compared favorably with that made by the regular method from similar milk.

The reduction in make time to 3 to 3½ hr. saved approximately half the time required by regular method. High, as well as low, temperature curing seems to be satisfactory for this type of cheese from pasteurized milk.

M12. Determination of free amino acids in Cheddar cheese by one-dimensional paper chromatography. D. H. BULLOCK, O. R. IRVINE, and W. H. SPROULE, Ontario Agricultural College, Guelph.

The one-dimensional buffered filter paper chromatographic method of analysis for the determination of amino acids, first described by McFarren, has been applied to the analysis of Canadian Cheddar cheese. Using 5 buffer-solvent combinations it has been possible to clearly separate 18 amino acids and amines from aqueous extracts prepared from either raw or pasteurized-milk cheese. As many as 17

different cheeses have been analyzed on one set of papers. The 5 buffer-solvent combinations were phenol saturated with an aqueous buffer at pH 12.0; collidine, at pH 9.0; m-cresol, at pH 9.0; m-cresol, at pH 8.4; and o-cresol, at pH 6.2.

This method showed greater precision than the two-dimensional method, in that spots could be better identified by a comparison with "standards" run on the same sheet. In some cases, increasing increments of the same extract were spotted as an aid in determining more accurately the presence and amounts of certain amino acids. On normal chromatograms there was little overlapping, and quantitative estimations could be made more accurately. These estimations were made by the maximum color density method with a photoelectric cell "scanner." Lesser amounts of solvents and filter papers were needed for this one-dimensional method although 5 separate chromatographic chambers were required.

M13. Formulation and stability of three new flavor varieties of Cheddar cheese spread for use by the Armed Forces. R. I. MEYER and J. M. MCINTIRE, QM Food and Container Inst., Chicago, Ill.

The Army formulation differs from that of usual commercial cheese spreads by the higher fat level (45%), lower moisture level (37%), and the elimination of whey and other ingredients containing lactose. These alterations have provided good spreadability and excellent nutrition without the deteriorating effects of significant levels of carbohydrate, which when present result in reduced keeping quality.

Employing the plain Cheddar cheese spread as a base, optimum levels of addition of a liquid smoke preparation, caraway seed or caraway oil, and canned sterilized pimientos in pureed form were determined with the aid of sensory evaluation tests carried out for a period of 1 yr. on samples stored at 40°, 70°, and 100° F. Evaluation of the averaged rank orders of the samples at the end of 1 yr. by temperatures between flavors showed a pattern of preference in the following order: 1) pimiento, 2) caraway, 3) smoked, 4) plain. All ratings were in an acceptable range after the end of 1 yr. storage at 100° F.

Other data collected included the per cent of free fatty acids, peroxide values, and pH values during the storage period. The relationship of the chemical and sensory evaluation tests will be discussed.

M14. Free fatty acids produced during Blue cheese ripening. H. A. MORRIS, J. J. JEZESKI, W. B. COMBS, and S. KURAMOTO, Univ. of Minnesota, St. Paul.

Determinations of fatty acids by partition chromatography were made on Blue cheese manufactured from variously treated milks. In raw milk cheese no caproic and higher acids appeared until 4 mo. Butyric acid did not appear until after 7 mo. These cheeses developed characteristic flavor between 9 and 11 mo. In the better cheese the caproic and higher acid values (av. M.W. 228) averaged 8.52 mg/g cheese. Butyric acid averaged 0.74 mg/g cheese. Homogenized-raw-milk cheese initially (after salting) contained caproic and higher acids but no butyric acid. Butyric acid was detected at 4-5 mo. when the flavor was the most acceptable. The caproic and higher fraction amounted to about 20 mg/g cheese.

The production of fatty acids during ripening in the homogenized heat-treated-milk cheese was similar to, but slightly lower than, the homogenized-raw-milk cheese. Pasteurized-homogenized-milk cheese did not develop free caproic and higher acids until 50 days. Butyric acid was detected at 4 mo. The best flavor was obtained at 5 mo. At this time the average caproic and higher acid value was 24.93 mg/g cheese and the butyric acid averaged 0.77 mg/g cheese.

M15. HTST pasteurization of ice cream mixes. J. TOBIAS, O. W. KAUFMANN, and P. H. TRACY, Univ. of Illinois, Urbana.

Processing of ice cream mixes in rapidly heating equipment with no intended holding time requires the knowledge of the lowest temperature giving comparable results to those obtained by accepted pasteurization methods. The flow diversion valve control may be set at this temperature even though the product is processed at much higher temperatures at the option of the operator.

Using *Micrococcus* sp. MS 102 as a test organism, the temperature giving equivalent destruction to pasteurization at 155° F. for 30 min. was found to be 187.2° F. The mixes were processed in the Roswell heater, which had an estimated effective holding time of 0.8 sec. The effective holding time is defined as the summation of the lethal effects of heat-up time, discharge time, sample collection time, and cooling time, expressed as hold at processing temperature.

From consideration of the z values of the test organism and those of *Mycobacterium tuberculosis*, it became evident that heating to 187.2° F. contained a smaller margin of safety than accepted pasteurization standards. Calculations of lethalities in terms of *Myc. tuberculosis* revealed that in order to maintain the level of safety margin presently afforded the processing temperature should be 194° F. with an effective hold of not less than 0.8 sec.

M16. A critical study of 2,6-dichloroquinonechloroimine as an indicator in the phosphatase test. R. W. HENNINGSON and F. V. KOSIKOWSKI, Cornell Univ., Ithaca, N. Y.

Solubility, time required for maximum color development, intensity of color produced, and chemical stability were studied for 2,6-dichloro-

quinonechloroimine (CQC) and 2,6-dibromoquinonechloroimine (BQC) as applied to the phosphatase test. CQC proved to be more soluble than BQC in absolute methanol, absolute ethanol, and 95% ethanol. Maximum color was produced more slowly with CQC than with BQC in both the Sanders-Sager test and the Cornell test, especially at the lower levels of phenol concentration. This maximum color was usually produced within the time limits set for each method and presented no particular problem.

Intensity of color produced with CQC was of the same order in the Sanders-Sager test when compared to BQC but was greater in the Cornell test. The chemical stability during storage of CQC and BQC was high when in the powder form. The stability of CQC in solution was much greater than that of BQC. The data indicate that CQC is potentially superior to BQC for use in the phosphatase test.

M17. Observations on raw milk quality before and after conversion to bulk tank pick-up at the farm. H. V. Atherton, Univ. of Vermont, Burlington.

A long-term study of patron deliveries to two local milk plants has made it possible to compare certain aspects of raw milk quality before and after conversion to bulk tank pick-up at the farm. The findings do not agree with many of the published results.

Approximately 175 flavor comparisons have been made between deliveries in 1952 and 1954. Feed flavors predominated in both years. Defects noted with bulk tanks in the 1954 study were not as serious as those encountered in 1952 under can cooling. Samples scoring 38 or more increased from 60% of the total in 1952 to 77% in 1954. A decline was noted in the incidence of unclean, rancid, and high-acid flavors. Despite this general improvement, nearly half of the deliveries sampled in 1954 scored lower than the comparable deliveries in 1952.

Total plate, thermoduric, and psychrophilic counts showed some improvement for the samples as a whole, but differences were seldom large and one-third of the farms have delivered higher count milk since converting to bulk tanks. Protein stability and creaming ability have been lowered in many instances since tank truck pick-up has been adopted.

M18. Butterfat tests of milk from farm bulk tanks. Alec Bradfield and P. E. Gotthelf, Univ. of Vermont, Burlington.

Previous study of weigh can sampling had shown that 50% of the sampling was in error and that 66% of the errors were in favor of the farmer. It was also indicated that high-fat milk was more likely to exhibit errors than low-fat milk. There seems to be no evidence that a producer's test will change when he shifts from can to bulk handling if he was getting the correct test under the can system.

Twenty-four lots of milk, 19 Holstein and 5 Jersey, were examined. Each lot was split, one half being handled in cans and the other in bulk tank. The milk handled through the weigh can was thoroughly stirred before sampling to insure complete mixing, and the bulk tank agitator was operated for 5 min. before sampling. The average difference in test between the cans and the tanks was 0.019%. None of the Holstein milk showed a difference of more than 0.05% and the Jersey milk not over 0.07% difference.

The amount of agitation necessary for complete mixing in a bulk tank was found to be 1 min. with agitator operating at 29 r.p.m. Only 4 lots out of 24 failed to be completely mixed in 30 sec.

M19. Study of several methods for the determination of total solids in fluid milk. W. R. Davey and Stuart Patton, The Pennsylvania State Univ., University Park.

A survey of several procedures involving the determination of moisture or total solids in fluid milk was made. Methods included hydrolysis of acetyl chloride, Karl Fischer titration, refractive indices on milk-glycerol mixtures, certain modifications of the Chemical Oxidation Demand test commonly used on dairy wastes, rapid drying of multiple samples with the Brabender Semi-Automatic Moisture Tester, turbidity tests, and colorimetric procedures.

The most promising procedures involved the use of the refractometer and Brabender Tester, and the C.O.D. method. If modifications of the refractometer and Brabender Tester could be made, more accurate readings with these instruments would be possible. Results of duplicate determinations made on 45 samples by the Brabender Tester when compared to the Mojonnier results showed an average variation of $\pm 0.148\%$. Twenty of the 45 samples showed variations of 0.10% or less, and 31 of the 45 samples showed variations of 0.20% or less. The C.O.D. method with appropriate modifications was simple to perform and showed good accuracy. An average of duplicate determinations on 30 milk samples when compared to the Mojonnier results showed an average variation of $\pm 0.174\%$.

M20. Effects of age upon the viscosity of whole milk. C. H. Whitnah, W. D. Rutz, and H. C. Fryer, Kansas State College, Manhattan.

Each Ostwald viscometer used in this study was mounted in a metal frame and placed in a water bath stirred by air bubbles. The temperatures were manually controlled with the aid of Beckman thermometers. Flow times were measured to 0.01 sec. with stop watches.

The milks used in this study were pasteurized 30 min. at 62° C. and then cooled either at once, or after being put thru a homogenizer at pressures ranging from 50 to 3,500 p.s.i. The age of each milk was the interval in hours from

the time of cooling until the time the viscosity was measured. The milks were stored at 4° C. until viscosities were measured at temperatures ranging from 4 to 44° C. Ages ranged from 0.3 to 300 hr.

At each temperature straight lines were fitted by the method of least squares to eight pairs of values for viscosity and log age. The slopes (b in the equation, viscosity $= a + b$ log age) of these lines were as follows:

Temp. °C.	4	8	18	22.7	29	34	39	44
b	182	155	112	94	59	37	18	10

A chart was prepared for correcting the viscosity of milk from any age at which it was measured to any other desired age.

M21. Some properties of "subnormal milk." W. A. KRIENKE, L. E. MULL, and E. L. FOUTS, Florida Agr. Expt. Sta., Gainesville.

"Subnormal milk" is lower than normal in butterfat content associated with conditions of inadequate roughage in an otherwise nutritionally balanced ration. Feeding practices to produce and correct it will be presented in another paper from this Station (P75).

Subnormal milk was produced by each of the 10 cows (Jerseys and Guernseys) receiving the special ration; butterfat tests lowered by 0.5-2.2%. Solids-not-fat values were lowered only slightly, resulting in an abnormal relationship of fat to solids-not-fat.

Some unexplained but highly important change in the proteins (casein) is evidenced by the fact that "slow" and "fast" cultures used in cottage cheese trials resulted in total loss and in inferior cheese of low yield, respectively, for blended subnormal skim as compared to fair and to excellent cheese, respectively, for blended control skim. Individual cow batches varied greatly in firmness of coagulum and in quality of cheese. Curd tension values on the whole milks paralleled these conditions, ranging from about 8.0-60.0 (more than half below 30) as compared to about 65 for control samples.

Total solids by the formula $TS = \frac{CL}{4} + 1.2\ F$ agreed with Mojonnier values. Subnormal milks were normal with respect to freezing point values.

M22. An evaluation of a centrifugal method for determining the efficiency of homogenization. D. A. SEIBERLING, The Ohio State Univ., Columbus.

Several tests for efficiency of homogenization have been suggested that rely upon centrifugation to induce fat separation in the homogenized sample. This investigation indicated that a method proposed by Maxcy (1950), utilizing an International Centrifuge, was rapid, simple, sensitive, and not unduly subject to errors of human judgment. This test was applied to samples of various ages with equal success and thereby provided an indication of homogenization efficiency under conditions prohibiting the use of gravitational methods.

A modified method, following the same procedure but utilizing a deep pocket Babcock Centrifuge, was shown to yield an accurate indication of homogenization efficiency. It can be conducted with equipment found in the average plant laboratory. Such a method appears applicable as a control test in that results can be obtained quickly.

A comparison was made of Maxcy's method, the modified Babcock Centrifuge method, the U.S.P.H.S. gravitational method, and Farrall's Microscopic Index method.

M23. Analysis of milk for free volatile fatty acids. F. V. KOSIKOWSKI, Cornell Univ., Ithaca, N. Y.

Principles used in the total volatile acid procedure of cheese (*J. Dairy Sci.*, **29**: 861. 1946) were applied to milk with certain modifications. Direct distillation of homogenized raw milk produced reliable volatile acid values. To observe the nature of free fatty acids in rancid milk, a recently described paper chromatographic technique (*Proc. 13th Intern. Dairy Congr.*, **3**: 1250. 1953) was applied to the titrated distillates from the total volatile acid procedure above. Fatty acids in the distillates were converted to their ethylamine salts and subjected to single dimension solvent runs with butanol.

Significant levels of free total volatile fatty acids were observed in homogenized raw milk. The influence of homogenization pressures, incubation periods, and temperature conditioning of milk was well defined. Typical values were on the order of 1.0 to 4.0 ml. N/10 NaOH/100 g. milk. Individual fatty volatile acids in rancid milk were observed on chromatograms as discrete blue areas against a yellow background. Among fatty acids identified were butyric and caproic.

The coupling of the direct distillation method for total volatile fatty acids to a chromatographic procedure for individual acids in milk should serve a useful function in future lipase and rancidity studies of milk.

M24. Isolation and identification of δ-decalactone as the compound responsible for the coconut-like flavor of butter oil. P. G. KEENEY and STUART PATTON, The Pennsylvania State Univ., University Park.

During the early storage period of certain fat-containing dairy products a flavor defect described as coconut-like develops. The defect is always evident in stored butter oil, dry whole milk and cream products, canned sterilized milk, and evaporated milk. Although not of an unsavory character, its presence in these products renders them readily distinguishable from fresh fluid milk.

The flavor compound has been isolated from butter oil and identified as δ-decalactone (the lactone of 5-hydroxy decanoic acid). A brief outline of the isolation procedure follows: 18 l. of heat-activated butter oil was extracted continuously with methanol for 2 days. The extractable fatty residue was chromatographed on alumina, and the lactone containing fraction was then agitated several hours with a dilute solution of sodium carbonate. After removal of the neutral material the hydroxy fatty acid salts were acidified and the reesterified lactone was extracted with ether.

The infrared spectrum of the unknown compound was identical with that of 5-decalactone. Additional evidence as to the identity of the compound was obtained by paper chromatography of the hydroxamic acid derivative.

M25. Prevention of sunlight flavor in milk by removal of riboflavin. H. J. VELANDER and STUART PATTON, The Pennsylvania State Univ., University Park.

Removal of riboflavin from milk by passage through Florisil prevented development of sunlight flavor. Adding riboflavin back to samples depleted of the vitamin in this manner restored susceptibility to the off-flavor. Intensity of sunlight flavor in both nonfat milk and homogenized milk was correlated positively with riboflavin content through a range of from 0 to 25 mg. of riboflavin per qt. In riboflavin-free homogenized milk, exposure to light produced oxidized but not sunlight flavor.

Microbiological assays indicated a loss of methionine as a result of exposing milk to sunlight. This loss was most easily detected in thin films of milk.

M26. The isolation and identification of the carbonyl compounds resulting from the oxidation of butter oil. E. G. HAMMOND and E. W. BIRD, Iowa State College, Ames.

Butter oil was oxidized at 40° C. until it had a tallowy and painty flavor. The carbonyl compounds present were separated from the butter oil by means of Girard's reagent T and then converted into 2,4-dinitrophenylhydrazones. The 2,4-dinitrophenylhydrazone derivatives were separated into several fractions on the basis of their solubility in petroleum ether, alcohol, chloroform, and nitromethane. The components of the various solubility classes were separated further by means of column chromatography. Methods for the paper chromatography of the various fractions have been worked out. By means of the ultraviolet spectra, R, values, colors produced by alkali, and solubilities of the 2,4-dinitrophenylhydrazones, it has been possible to assign probable structures to several of the compounds.

M27. Breed and individual variations in the specific protein constituents of milk. G. D. ROLLERI, B. L. LARSON, and R. W. TOUCHBERRY, Univ. of Illinois, Urbana.

Two-day composite samples of milk from the 5 major dairy breeds were taken near mid-lactation and were analyzed by a quantitative electrophoretic procedure to determine the differences between and within breeds for the total and specific constituents of the casein and the serum proteins. Five to 7 cows were included for each breed.

Holstein milk contained significantly less total casein (2.38 g. per 100 ml. skimmilk), α-(1.58 g.), and β-(0.60 g.) but significantly more γ-(0.20 g.) casein than milk from the 4 other breeds. Ayrshire milk (2.65 g. casein per 100 ml. skimmilk) contained significantly less γ-(0.08 g.) casein than the 4 other breeds and significantly less α-(1.71 g.) casein than the Guernsey, Jersey, and Brown Swiss breeds. These 3 breeds did not differ significantly from each other in total casein (2.89, 2.72, and 2.78 g. per 100 ml. skimmilk, respectively) or in any of its constituents.

Even though individuals varied considerably, the breeds did not differ significantly in the total amount of serum proteins (0.51 to 0.66 g. per 100 ml. skimmilk) or in any of its constituents (immune globulins, α-lactalbumin, β-lactoglobulin, and serum albumin). Usually, however, the constituent averages tended to be the lowest or highest for the Holstein breed.

M28. Water-insoluble acid content of farm-separated cream produced in Kentucky. T. R. FREEMAN, L. A. RICHARDSON, and J. O. BARKMAN, Kentucky Agr. Expt. Station, Lexington.

For 1 yr. approximately 20 individual producer shipments of cream were sampled monthly at each of 3 buying stations. Composite lots of cream also were sampled and held at 50°, 65°, and 80° F. and at atmospheric temperature for 24- and 48-hr. periods. Analyses for water-insoluble acid (WIA) content were made by Hillig's rapid method.

Mechanical separation produced cream with lowest, and water-dilution separation with highest, WIA content. Cream stored on the farm under artificial refrigeration had lower WIA content than unrefrigerated cream or cream cooled in cellars, caves, springs, etc. The older the cream at time of delivery, the higher was its WIA content. The increase in WIA after delivery to the cream station was directly related to storage temperature during this time. Lowest WIA values were observed in May-July and highest in Dec.-Feb. Average WIA values observed at the 3 stations were about the same. No specific flavor defect in the cream was found to be closely related to high WIA values, although 45% of the "cheesy" samples and 35% of the "rancid" samples had WIA values over 500. It was found that WIA values were inversely related to the fat content of the cream samples.

M29. The separation of the free fatty acids of mold hydrolyzed butterfat by partition and displacement chromatography. S. KURAMOTO, J. J. JEZESKI, and H. A. MORRIS, Univ. of Minnesota, St. Paul.

The carrier displacement separation of the fatty acids of butterfat after saponification has been described (*Proc. Am. Oil Chem. Soc.*, Oct., 1954). This procedure was used in estimating the end products resulting from the action of *P. roqueforti* lipase on butterfat.

Butter oil was separated into liquid and solid fractions at 25° C. These fractions were saponified and the fatty acid distribution determined. The two fractions were subjected to lipase action with mycelia of *P. roqueforti* as the source of enzyme. An initial separation into a C_4 and a C_5 and higher fraction was performed by partition chromatography on silicic acid gel, and subsequent separation of the higher acids was accomplished by carrier displacement analysis.

The results indicate that the component fatty acids of the liquid and solid fractions do not vary markedly. The only variation appeared to be in a greater proportion of the lower acids (C_4 to C_9) in the liquid fraction, The greatest activity of the mold appeared to be on the liquid fraction and the least activity on the solid, with the unfractionated butter oil giving intermediate results. Although it was observed that the liquid fraction promoted the greatest activity, the mole fraction of acids liberated was about the same in both liquid and solid butter oil samples.

M30. The effect of several organic and inorganic acids on the heat precipitation of calcium hardness from water. D. A. EVANS and G. H. WATROUS, JR., The Pennsylvania State Univ., University Park.

The precipitation of temporary water hardness as calcium carbonate by temperatures of 160° F. or over is a problem where hot water sterilization is necessary in hard water areas. Although it long has been recognized that acidification of such water would reduce or eliminate each precipitation, specific data is lacking on the critical levels of various organic and inorganic acids needed with water of varying hardness, heated to temperatures usual in dairy plant sanitizing procedures.

In the present study, water showing from 5 to 20 grains calcium hardness was adjusted to pH values ranging from 3.0 to 6.0 with hydrochloric, phosphoric, acetic, gluconic, levulinic, and hydroxyacetic acids. After acidification, samples were held at temperatures of 165° F. for 10 min. and 180° F. for 10 min. Residual calcium, in the water samples, was determined by oxalate precipitations.

The concentration of acid required to prevent precipitation appears to be a function of calcium hardness concentration, the particular acid employed, the ionization constant of the acid, the solubility of the salt formed and the heating temperature and time. Observations with the acids studied indicate that possibly hydroxyacetic and gluconic acid, at certain concentrations, may cause more corrosion than the other acids investigated.

M31. Direct chromatographic determination of lactic, pyruvic, and C_1 to C_6 fatty acids in biological materials. MARK KEENEY, Univ. of Maryland, College Park.

A method will be described by which the acids are separated and determined without previous distillation or extraction of the acids from the sample. An indicator is used in the chromatographic column so that the movement of acids on the column may be followed visually. Application of the method to synthetic acid mixtures has indicated quantitative separation and recovery of the acids. Use of the method in the study of dairy products and rumen fluid will be discussed.

M32. Acids formed by the high-heat treatment of milk. C. V. MORR, I. A. GOULD, and W. J. HARPER, The Ohio State Univ., Columbus.

Raw and heated skimmilks were analyzed for the presence of individual free organic acids by a modification of the silica gel chromatographic method of Bulen *et al.* (1952). Raw milk showed the presence of small amounts of butyric, acetic, pyruvic, formic, lactic, and 4 other unidentified acids. The total concentration of these 9 acids ranged from 80 to 200 ue/50 g. Rather large variations in concentrations of individual acids were observed in various samples.

Milk heated at 100° C. in sealed containers generally showed increases in the concentration of all these acids after 30 to 60 min. of heating. The increase in formic acid was highest throughout the 6-hr. heating period. After 6 hr. the values for formic acid ranged from 240 to 280 ue/50 g.; acetic acid ranged from 60 to 175 ue/50 g.; and lactic acid values ranged from 21 to 45 ue/50 g. of milk.

The addition of phosphates to the milk resulted in a more rapid rate of production of each of the various acids. In both normal and phosphate-treated milks, the increase in formic and acetic acids coincided with the increase in the degree of "browning" of the sample.

M33. Synthetic vitamin K and oxidized flavors in milk.[1] M. RECHCIGL, JR., V. N. KRUKOVSKY, and L. H. SCHULTZ, Cornell Univ., Ithaca, N. Y.

A study was made to learn whether or not the feeding of menadione and synkayvite, fat and water soluble forms of vitamin K, respectively, to cows secreting milk in which the reactions resulting in the development of oxidized flavors are readily catalyzed by copper, or their

[1] The assistance of Hoffman-LaRoche, Inc., in furnishing the vitamin K is acknowledged.

addition directly to milk, would result in the retardation or prevention of oxidative processes. The results indicate that neither the daily feeding of 100 and 200 mg. menadione and synkayvite, respectively, per cow during a 10-day trial nor their addition to milk at the rate of 10 mg/l (milk becomes unpalatable when menadione is added in excess of 10 mg.) resulted in the prevention of oxidized flavors. Moreover, the feeding of both menadione and synkayvite apparently stimulated the fishy flavor in the milk. The added menadione was quantitatively removed from milk by shaking-out the vitamin with Skellysolve B in 1:1 ratio. Its concentration in the solvent was determined by application of Martinson and Meerovich colorimetric method, involving interaction of aniline with 2 methyl-naphtha-quinone. Menadione was not detected by this method in milk from menadione-fed cows.

M34. Organoleptic study of oxygenated and copper-treated milk prior to pasteurization. V. N. KRUKOVSKY, Cornell Univ., Ithaca, N. Y.

In this study the occluded air in the milk from cows on the rations which produce unstable milk was replaced with oxygen passed through the milk at 20-25° C., by means of a gas dispersion tube for 3-5 minutes, prior to the addition of 0.1-1.0 mg. Cu/l and holding at 5°, 20°, and 61° C. The observations have shown that the rate of ascorbic acid oxidation with added Cu as a catalyst increased directly with the amount of Cu and the temperature of milk. Pasteurization of oxygenated milk with 0.25 mg. Cu at 61° C. for 30 min. resulted in the complete depletion of milk of the total vitamin C content and the prevention of the reactions which produce oxidized flavors during 10 days storage at 2-5° C. These reactions were induced again, however, and the metallic and fishy flavors were developed by the addition of ascorbic acid to such milk. The rate of oxidation of added ascorbic acid was about the same as that of oxygenated milk treated with Cu after the heat treatment, but greater than that in control pasteurized and copper-treated milk.

M35. The occurrence of rancidity in milk from pipeline milkers.[1] G. W. GANDER, Cornell Univ., Ithaca, N. Y.

The effect of pipeline milkers in inducing rancidity was studied. The acid degree value was used as a measure of the extent of lipolysis and, in addition, some samples were tested organoleptically. The activation of the lipase system in a pipeline milker with 5 risers was studied under practical conditions. Samples were removed from the pipeline after each riser.

To determine the extent of rancidity in milk handled by pipeline milkers, samples were collected from 21 producers in a selected area of New York state at 3 periods during 1953-54. Fourteen of these producers were using pipeline milkers. The pipeline systems were divided into 2 groups: those with and those without risers. Samples from 7 producers using conventional bucket milkers served as controls. Acid degree values of the controls fell within a narrow range, whereas wide variations were found in the pipeline systems containing risers. The pipeline systems without risers did not show such wide variations.

M36. Development of oxidized flavors in unhomogenized milk and homogenized milk. E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y.

Should the Department of Dairy Industry homogenize all its market milk to retard the development of the oxidized flavors? In order to answer this question a survey was started on Dec. 20, 1954, and continued to June, 1955.

The score card employed was: –, no oxidized flavors; ?, indication of oxidized flavors; 1, slight oxidized flavors; 2, distinct oxidized flavors; 3, strong oxidized flavors; and 4, very strong oxidized flavors. The milks were scored on the 3rd, 5th, and 7th days. After 40 days, in the unhomogenized samples on the 5th day 3 milks scored 2 and 3 milks scored 3. On the 7th day 14 milks scored 2 and 10 milks scored 3. In the homogenized samples all milks scored – or ?. It is considered that 2 is not acceptable to some consumers and that 3 is objectionable to most consumers.

M37. The effect of mineral fortification on lipase activity in pasteurized milk. R. A. REISFELD, W. J. HARPER, and I. A. GOULD, The Ohio State Univ., Columbus.

Prior work has shown that the free fatty acid content of commercial vitamin-mineral fortified pasteurized milk (milk containing added iron, iodine, vitamins A, D, and B complex) was found to increase during storage at 40° F. (Gould and Harper, 1954). The addition of ferric ammonium salt to the milk prior to processing was found to be responsible for the apparent "lipase activation" after homogenization and pasteurization.

Continuation of this study was made to determine the role of the ferric ammonium citrate in protecting the milk lipase during pasteurization (HTST). Various iron and citrate salts were added to determine what part of the ferric ammonium citrate salt was involved in the protection of the lipase. Results revealed that the addition of ferric iron salts protected the milk lipase against heat treatment, whereas citrate salts did not protect the lipase to heat inactivation at normal pasteurization temperatures (164° F. for 16 sec.).

Study was made also of the effect of the sequence of processing on the protective effect of ferric iron. Results revealed that complete

[1] Supported by the Rite-Way Dairy Division, Package Machinery Co., East Longmeadow, Mass.

pasteurization before homogenization eliminated the protective effect of ferric iron and that homogenization just prior to holding (16 sec.) minimized the effect of the ferric iron.

M38. Observations on the extent of lipolysis in raw milk supplies as related to various milk handling procedures. E. L. THOMAS, A. J. NIELSEN, and J. C. OLSON, JR., Univ. of Minnesota, St. Paul.

A survey was made of the extent of lipolysis occurring in milk secured from selected farms having different types of milk handling equipment. Combinations included: *(a)* handling in cans; *(b)* handling in bulk tanks (no pipeline); *(c)* handling in pipeline milker and bulk tank; and *(d)* handling in pipeline milker and cans. The extent of lipolysis was estimated by the acid degree value of fat obtained by the B.D.I. detergent extraction method of Thomas *et al.* (*Am. Milk Rev.*, **17** (1): 50. 1955.)

Results showed that excessive lipolysis occurred most frequently in milk from farms employing pipeline milker and bulk tank combinations. Detailed studies revealed that the principal factor promoting lipolysis was apparently excessive air intake combined with agitation in various sections of the pipeline milking equipment, including the milker unit. Plugging air ports and tightening loose connections resulted in marked reduction of lipolysis and eliminated detectable rancidity. These observations agreed with results of laboratory studies by Kelley and Dunkley [*J. Milk Food Technol.*, **17** (10): 306. 1954.] Handling of milk in bulk tanks per se had no significant influence on the acceleration of lipolysis even under conditions of every-other-day pickup.

M39. The relationship of processing variables to the feathering of coffee cream. O. B. CURLEY, I. A. GOULD, and D. A. SEIBERLING, The Ohio State Univ., Columbus.

Coffee cream was processed under varying conditions of pasteurization temperature, holding time, and homogenization temperature and pressure. Several different homogenizer valves representative of those in commercial use were used at pressures which ranged from 300 to 1,200 lb.

The feathering properties of the cream samples were determined by adding 1-ml. portions of cream to test tubes containing coffee at 205°-210° F. Various amounts of citric acid or sodium citrate had previously been added to this coffee. This technique provided quantitative as well as qualitative indications of the cream sample's susceptibility to feathering. It was applied immediately after processing and after periods of 1 to 3 days.

Under laboratory conditions, the efficiency of homogenization and the feathering properties of coffee cream were found to be directly related to homogenizer valve design and homogenizing pressure. In producing a cream resistant to feathering, consideration should therefore be given to the type of homogenizer used, its location in the process, and processing time-temperature relationships.

M40. The stability and role of aureomycin in milk.[1] K. M. SHAHANI, I. A. GOULD, H. H. WEISER, and W. L. SLATTER, The Ohio State Univ., Columbus.

The heat stability of aureomycin in milk has been investigated. The Disk Assay Method has been standardized to determine 0.075γ/ml. Aureomycin (0.2 to 0.4γ/ml) was added to raw milk, 0.1 *M* monopotassium phosphate, and to water. The products were heated at 143° F. and 160° F., and the rate of aureomycin inactivation was determined after 10, 20, 30, 40, and 50 min. of heat treatment. There was a direct relationship between the temperature of heating, length of holding time, and the amount of aureomycin inactivation. The per cent loss of aureomycin was least in milk, somewhat greater in buffer, and greatest in water.

Aureomycin was added to raw milk, buffer, and to water. Half of each of these products was pasteurized. The samples were stored at 2-4° C. and assayed every week for 3-5 wk. The storage loss of aureomycin potency was much faster in raw samples than in the pasteurized ones. Presumably, milk solids exert a protective effect upon the antibiotic during heating and storage.

Preliminary observations indicate that aureomycin partially inhibits the lipase activity in raw milk products.

[1] Supported by a grant from the U. S. Public Health Service (National Institute of Health) and by funds from the Ohio Dairy Products Research Fund.

M41. A test for keeping quality of pasteurized milk. E. A. DAY and F. J. DOAN, The Pennsylvania State Univ., University Park.

Evidence indicates that dye reduction tests, employing either resazurin or p,p′-diphenylene-bis-2-(3,5-diphenyltetrazolium chloride) (DBDTC), under vacuum, are the most satisfactory of the several methods examined for conveniently determining the keeping quality of bottled pasteurized milk without resort to bacteriological procedures. A noticeable change to a definite pink in stored milk containing the dyes after 4 hr. incubation at 37° C. is predictive of psychrophilic bacterial spoilage within a 3- to 4-day period. The standard resazurin test was modified by evacuating the test tube at 3° C. and 15 mm. pressure for 3 min., after which it was sealed and incubated at 37° C. DBDTC was added to 5 ml. of milk at the rate of 0.5 ml. of a 0.2% aqueous solution. Evacuation and incubation were carried out as described for resazurin. DBDTC is advantageous in that it changes from colorless to colored when reduced to the diformazan, the reaction

being irreversible. A procedure has been developed whereby the diformazan may be extracted and the degree of reduction measured colorimetrically. Evacuation is critical in these tests, but it may be accomplished with a laboratory aspirator using a test tube fitted with a 1-hole rubber stopper and a clamp on the rubber evacuation tube.

M42. Some characteristics of the natural antibacterial properties of milk. A. G. WOLIN and F. V. KOSIKOWSKI, Cornell Univ., Ithaca, N. Y.

During recent disc assays studies for antibiotics in milk (*J. Dairy Sci.*, **35**: 533) small zones of inhibition were noted in some raw milk samples known not to contain antibiotics, sulfa drugs, or quaternary ammonium compounds. This phenomenon, recognized in the past by other criteria, was ascribed to the natural bacterial inhibitory properties of milk. It was possible, in studying this property of milk by disc assay, to increase zone size and sensitivity by substituting dried milk discs for saturated paper discs. Milks tested were lyophilized and made into a pill, which was then placed on agar plates seeded with the test organism. Bacteria such as *Bacillus subtilis*, *Streptococcus faecalis*, and *Escherichia coli* displayed marked sensitivity to the natural inhibitory properties of milk and were suitable as test organisms.

Generally, raw milk produced two closely defined zones against the bacteria. An initial clear zone, indicating no growth, appeared first after 12 to 24 hr. of incubation at 72° F. A secondary zone then appeared, which was characterized by the disappearance of colonies, like that associated with bacterial lysis. The size and nature of these zones varied seasonally, and milks from mastitic cows reacted differently from milk of normal cows.

M43. Observations on bacterial growth stimulants present in pancreas tissue. W. E. SANDINE, L. HANKIN, M. L. SPECK, and L. W. AURAND, N. C. State College, Raleigh.

Earlier work (Kizer *et al.*, *J. Dairy Sci.*, **37**: 634. 1954) has demonstrated the presence of stimulatory substances associated with certain proteolytic enzymes. Aqueous extracts of fresh pancreas tissues also have been found to be stimulatory for *Lactobacillus casei* and *Streptococcus lactis* in presumably nutritionally complete media, and for *S. lactis* in milk.

Paper chromatograms of pancreas extracts were bio-autographed on media containing either enzymatic or acid hydrolyzates of casein and seeded with *S. lactis* or *L. casei*. With both media, the same two active components were observed. Their activity, however, was much greater on the acid hydrolyzate medium. Since the two factors are active in the presence of tryptic digest of casein, they presumably are different from strepogenin. Bio-autographs made with litmus milk agar and *S. lactis* also detected these active components, as well as a third which was not observed on bio-autographs with other media. Liver fraction L and yeast extract also apparently contained one of the active components which was present in pancreas extracts.

Present work is concerned with the identification of these stimulatory factors and their relationship to other growth stimulants.

M44. The application of the 2,3,5-triphenyltetrazolium chloride test for inhibitory substances in milk. C. E. NEAL and H. E. CALBERT, Univ. of Wisconsin, Madison.

The 2,3,5-triphenyltetrazolium chloride (TTC) test, with *Streptococcus thermophilus* as a test culture, has been employed to test herd milks for the presence of inhibitory substances. When 223 herd samples were tested by this method, 2 samples showed complete inhibition to the test, and 30 indicated lesser degrees of inhibition. Milk samples that showed inhibition were checked for acid development by the test culture. There was good correlation between inhibition of acid development and the lack of development of color in the TTC test.

Milk from individual cows considered to be normal and free from mastitis was tested. No variation between cows could be detected in normal milk by the TTC test. Milk from cows with mastitis caused various degrees of inhibition to the test. In some instances complete inhibition occurred before the cow was treated for mastitis.

Sanitizers, such as quaternary ammonia compounds, the iodine types, and hypochlorites, could not be detected at low concentrations in raw milk. The TTC test as performed can be considered a test for antibiotics, and, in some instances, a test for the detection of abnormal milk caused by mastitis.

M45. Microbiological fermentation of dairy wastes.[1] J. E. EDMONDSON and A. R. BRAZIS,[2] Univ. of Missouri, Columbia.

Because of the growing demand for control of pollution of surface waters by sewage and industrial wastes, a study was made of the effectiveness of treating dairy wastes with microorganisms to reduce the B.O.D. A survey of small dairy plants in Missouri showed that the average 5-day B.O.D. ranged from 270 to 6,200 p.p.m., with the majority in the upper range.

The dairy waste was treated by the fill-and-draw principle with a continuous recirculating trickling filter with sedimentation tank. Microorganisms that were found to effectively lower

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[2] Present address: Howard A. Taft Engineering Center, U. S. Public Health Service, Cincinnati, Ohio.

the B.O.D. of dairy wastes are: *Pseudomonas cyanogenes, Ps. putrefaciens* and *Ps. viscosa.* The following results were derived from this study: *(a)* Certain *Pseudomonas* species can be used effectively to treat dairy wastes, provided alkalinity is maintained and aerobic conditions for fermentation are present; *(b)* buffered nutrient broths can be added to dairy wastes to cause desirable fermentations by certain microorganisms present in the waste, thus resulting in a stable effluent; *(c)* dairy waste can be treated with moderate results by means of continuous pH standardization.

M46. Effects of ultrasonic waves on the bacterial flora of milk. J. A. ELLIOTT and W. C. WINDER, Univ. of Wisconsin, Madison.

Early experimental results showed a high ultrasonic destruction of lactic streptococci in skimmilk. Less destruction was obtained in whole milk. At this stage consistently accurate temperature control within the sample under treatment was questionable. A continuous-flow treatment cell was designed and constructed, which afforded more accurate temperature control. It was found that at low temperatures the destruction of bacteria in milk or skimmilk was poor. At sample temperatures above 40° C., results were erratic. It was suspected that the ambient temperature of the sample under treatment did not indicate actual temperatures reached in the focal zone of the ultrasound beam. The extent of localized heating was determined by measurement of phosphatase destruction, since ultrasonic vibrations alone do not destroy milk phosphatase. The phosphatase destruction curve revealed that localized heating did occur which could not be measured with thermometers or thermocouples. Subsequent studies indicated that the destruction of bacteria in milk with ultrasonic treatment can be attributed to thermal effects. In milk the ultrasonic energy is rapidly dissipated in the form of heat. Since ultrasonic generation of heat is very inefficient, pasteurization with this method appears to be impractical.

M47. HTST pasteurization for the control of psychrophilic organisms in plant water supplies. D. A. SEIBERLING and W. J. HARPER, The Ohio State Univ., Columbus.

A HTST water pasteurizer utilizing standard heat exchangers and self-operating temperature controllers was applied to treat water used for washing cottage cheese curd and for plant cleaning operations. Heating was accomplished by regeneration and direct steam injection to a temperature of 180° F. After holding for 20 sec., the water was cooled by regeneration to a temperature suitable for clean-up use, and by additional refrigeration to temperature suitable for washing cheese. Temperatures approaching 40° F. could be attained.

Application in one commercial plant troubled by psychrophilic organisms in its city water supply has enabled production of cheese having better keeping quality than previously attained with chemical sanitizers. The use of wash water of a uniform low temperature has been found to improve the uniformity and quality of the cheese curd.

M48. Interactive phenomena among several bacteria of dairy origin. W. C. VAN DER ZANT, Texas Agr. Expt. Sta., College Station.

Drops of one of the effector species *(S. marcescens, A. aerogenes, E. coli, S. faecalis,* and *S. liquefaciens)* grown in nutrient broth were placed on a nutrient agar plate with a 27-gauge needle in 4-6 rows with increasing concentration from left to right. After drying, drops of one test species *(M. flavus, M. candidus, M. freudenreichii, S. flava, G. tetragena, S. marcescens, E. coli,* and *A. aerogenes)* were placed on the plate, overlapping the effector species and increasing in concentration from bottom to top. Similar plates with separate drops were prepared as control. Changes in the number and size of the colonies in and beyond the overlap were recorded after incubation at 32° C. for 24 hr. The inhibitory action of *E. coli* and *A. aerogenes* was limited to a partial or complete reduction of growth of the test species in the overlap and seemed independent of the concentration of the test species. Mutual inhibition was evident with *S. marcescens, S. liquefaciens,* or *S. faecalis* against *E. coli* or *A. aerogenes.* The extent of inhibition depended on the ratio of concentrations of the species in the drop-pair. The diffusible and complete inhibition of *M. flavus, M. candidus, M. freudenreichii, S. flava,* and *G. tetragena* by certain concentrations of *S. marcescens, S. faecalis,* and *S. liquefaciens* was affected by the concentration of the test species.

M49. Effect of phospholipids and unsaponifiable matter on the flavor stability of milk fat. E. N. FRANKEL, L. M. SMITH, and E. L. JACK, Univ. of California, Davis.

Milk fat prepared by solvent extraction of cream and buttermilk exhibited a much longer induction period at 80° C. than the fat obtained from the same cream by churning. The results showed that the lipids associated with the fat globules in cream and removed from the fat during churning and filtration were responsible for the differences in apparent stability.

Although the addition of milk fat fractions rich in phospholipids to churned fat retarded the accumulation of peroxides, as determined by the ferric thiocyanate method, a brownish discoloration developed in the fat whose flavor characteristics were being determined. The addition of unsaponifiable matter to churned milk fat at 1% and 4% levels decreased its stability. However, when added to solvent-extracted fat, the unsaponifiable matter greatly prolonged the induction period. This suggested a very effective synergistic action between phospholipids

and some component(s) of the unsaponifiable matter of milk fat. The effect of some of the individual constituents of the unsaponifiable matter on the stability of milk fat has been studied.

M50. Characteristics of pure fats compared to the same fats as extracted from frozen desserts. C. L. BURTON, M. LOEWENSTEIN, and J. B. MICKLE, Oklahoma A & M College, Stillwater.

In conjunction with other work at this station, it seemed logical to determine if the process of extraction caused any change in the composition of the recovered fat as compared with the original. The following fats were used: 2 samples of butterfat, 2 of vegetable fat, and an animal fat. Each sample was divided into 2 lots; one was made into frozen dessert mix and the other served as a control. The fat from the mixes was recovered by the Mojonnier procedure and the following tests were run: Reichert-Meissl number, saponification number, iodine number, melting point, and refractive index.

One extracted butterfat sample had a higher melting point and iodine number than its control. The extracted sample of the other butterfat had a higher refractive index than its control. Both vegetable fat samples showed an increased refractive index when extracted from mix. One vegetable fat also showed an increased melting point as a result of extraction. The animal fat showed a decreased refractive index. In all other cases the variation between replicate extractions was equal to, or greater than, the variations obtained between the pure and extracted fats.

M51. Study of the effect of extraction methods on composition of fat taken from frozen desserts. J. B. MICKLE and M. LOEWENSTEIN, Oklahoma A & M College, Stillwater.

Preliminary work directed toward detecting the adulteration of butterfat in ice cream indicated that the composition of butterfat varied somewhat, depending on the procedure used to extract it from the ice cream. Accordingly, a study was made of the effect of certain extraction procedures on the composition of the recovered fat as measured by selected fat constants. Ice cream mixes were prepared with butterfat and with a vegetable fat (mostly cottonseed oil). The fats were then extracted from the mixes and the following determinations were made: saponification number, steam volatile fatty acids, free fatty acids, iodine number, and melting point.

Butterfats extracted by modified Babcock procedures were compared to the fat obtained by the Mojonnier ether extraction procedure. The butterfat extracted by the Illinois and Minnesota procedures did not appear to differ from that extracted by the Mojonnier. In general, the fat extracted by the Nebraska and Pennsylvania methods had lower iodine numbers and less volatile fatty acids than the fat obtained by the three other extraction procedures. The free fatty acid content of fat extracted by the Nebraska and Pennsylvania methods was much higher than it was for fat obtained by the other procedures.

M52. Improved fractionation and fat number determination for detecting butterfat adulteration. W. A. KRIENKE, Florida Agr. Expt. Sta., Gainesville.

The modified method requires only 25 g. fat. Reproducible fractions have been obtained by a procedure of seeding with fat crystals followed by brief stirrings at 30-min. intervals during a period of 4 hr.

Liquefied fat at 110° F. was cooled to 90° F. and fractionated. The solid fraction was returned to the 110° F. cabinet for a period of 24 hr. A portion remained solid at this temperature, though none had crystallized at 110° F. Successive fractionations of the liquid portions were completed at 80° and at 70° F. so that 5 fractions in all were obtained: *(a)* solidified at 80°, did not melt at 110° F., *(b)* solidified at 90°, melted at 110° F., *(c)* solidified at 80° F., *(d)* solidified at 70° F., *(e)* liquid at 70° F.; distributions, respectively, were approximately 9, 38, 23, 15, and 15%. All of each fraction was recovered by rinsing filter paper and glassware with warm petroleum ether.

Fat numbers obtained were slightly higher values than respective Reichert-Meissl numbers. Data on a limited number of samples, based on fat numbers, show possible detection of foreign fats in concentrations as low as 3.0%. Butterfat fractions by this procedure may be useful, also, in fundamental research involving nutrition and for product development.

M53. A modified hydroxamate method for determining the short-chain fatty acids in fats. W. F. SHIPE, Cornell Univ., Ithaca, N. Y.

A modification of the colorimetric method for measuring the quantity of hydroxamic acids has been developed. This method involves 2 steps: 1, the separation of the water-soluble from the water-insoluble hydroxamates; and 2, the partitioning of the water-soluble hydroxamates between water and a butanol-ethanol mixture. The initial water-soluble fraction contains the hydroxamates of butyric, caproic, caprylic, and capric acids. In the second separation most of the butyric and caproic hydroxamates remain in the aqueous phase, whereas the other two hydroxamic acids are extracted in the alcohol phase. The relative proportion of these 2 pairs of acids can be estimated by comparing the color intensity in the aqueous phase before extraction with the intensity after extraction.

This method can be used to distinguish between butterfat and other fats. The results to date indicate that this method has merit as a screening test for adulteration of dairy products with nonmilk fats.

M54. Studies on nonwashing of butter. A. H. WHITE and R. R. RIEL, Dairy Technol. Unit, Dept. of Agriculture, Ottawa, Can.

Churnings of washed and nonwashed butter were made in 29 creameries from split vats of cream of varying quality from June to Nov., 1953. The butters were stored at –5° F. and scored monthly for 6 mo. There was no significant difference in the flavor quality of comparable butters before and after storage. Neither cream quality nor storage was a significant factor statistically to the comparative keeping quality of the washed and nonwashed butters. Increases in curd content due to nonwashing ranged from 0.35 to 0.55% and averaged 0.53%.

There was no consistent pattern to the bacteria and mold and yeast counts of comparable butters, and no relationship was noted between microbiological content and flavor scores. Peroxide and fat acidity values were similar for both butters before and after storage; pH values of nonwashed butters were slightly lower than for washed butters and were mostly in the range of 7.0 to 8.0.

M55. Tocopherol content of the fat of dairy products as an index of adulteration. J. H. MAHON, CONSTANCE ANGLIN, and R. A. CHAPMAN, Food and Drug Laboratories, Ottawa, Can.

The tocopherol procedure, originally developed to detect vegetable fat adulteration of the fat of butter, has been extended to the analysis of fat of evaporated milk, condensed milk, whole milk powder, ice cream, and cheese. The tocopherol content of the fat from evaporated milk, condensed milk, whole milk powder, and Cheddar cheese is very close to that of butterfat produced at the same time of year. Factors affecting the reliability of this test are discussed. Examples are given to demonstrate the effectiveness of this procedure for detecting the adulteration of the fat of evaporated milk, condensed milk, and whole milk powder.

M56. Tortelli-Jaffe reaction for detecting marine fats in butterfat. J. H. MAHON and R. A. CHAPMAN, Food and Drug Laboratories, Ottawa, Can.

The Tortelli-Jaffe reaction has been investigated as a means of detecting the adulteration of butterfat with marine fats. The reaction consists of dissolving the fat in organic solvents and adding bromine. Optimum conditions for the test have been studied. Marine fats or hydrogenated marine fats yield intense green, blue, or purple colors, depending upon the conditions. Animal fats yield yellow colors.

M57. Acetic acid turbidity temperature as an index of butterfat adulteration. J. H. MAHON and R. A. CHAPMAN, Food and Drug Laboratories, Ottawa, Can.

This test is proposed as a rapid screening test to detect butterfat adulteration. Values for some typical fats are as follows: butterfat 30°, margarines 67-72°, lard 80°, tallow 86°, hydrogenated whale oil 77-87°, and hydrogenated herring oil 90-91° C. Adulteration of butterfat with 10% of these fats raises the A.A.T.T. value by 4 to 6° C. The range for genuine Canadian butterfats will be presented.

PRODUCTION SECTION

P1. Further studies on the influence of dietary calcium and phosphorus on the incidence of milk fever. J. M. BODA, Univ. of California, Davis.

A previous paper presented data indicating a lowered incidence of milk fever in cows fed a low-calcium, high-phosphorus prepartal diet. Since the rations employed were unpalatable and did not supply sufficient nutrients for desired gains before calving, trials have been conducted with milk fever-susceptible cows in an attempt to find a prepartal diet that would correct these deficiencies and still prevent milk fever.

Aged, high-producing Jersey cows were placed in a dry lot for at least 1 mo. before calving and individually fed a basic ration consisting of oat hay and a concentrate mixture of 40% ground barley, 30% rolled barley, 25% wheat bran, 5% cottonseed meal and monosodium phosphate. The amounts of oat hay and phosphate fed were varied in order to adjust the dietary calcium and phosphorus.

Two of 26 cows (5 with past histories of milk fever) fed a daily ration of 8 lb. of oat hay and 8 lb. of the concentrate mixture (1.5% supplemental sodium phosphate), 2 of 19 cows (9 with histories) receiving the same ration except for 5% phosphate supplementation, and only 1 of 23 cows (11 with histories) fed 5-6 lb. of oat hay and 10-12 lb. of the concentrate mixture (5% sodium phosphate) developed slight clinical symptoms of milk fever.

P2. Relative reactions of cattle and man to higher temperatures.[1,2] SAMUEL BRODY, Univ. of Missouri, Columbia.

Indian cattle are said to be heat-tolerant because they have higher evaporative cooling (sweat more) than do European cattle. Man is still more heat-tolerant because he sweats still more. Our data, however, show that at 80° F. the evaporative cooling in Cal/m^2/hr was

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[1] Data from Research Bulletins in the Environmental Physiology and Shelter Engineering Series of the Mo. Agr. Expt. Sta. Authors include S. Brody, H. H. Kibler, C. R. Blincoe, A. C. Ragsdale; also D. M. Worstell, H. J. Thompson, and R. G. Yeck of the USDA.

[2] Journal Series Paper No. 1520 approved by the director of the Mo. Agr. Expt. Sta. Thyroid activity data at various temperatures were obtained with the aid of the U. S. Atomic Energy Commission.

approximately 100 for resting lactating European cows, 60 for Indian cows, 20 for resting man. The greater evaporative cooling in cattle results partly from their greater respiratory vaporization: at 70° F. the respiratory vaporization in Cal/m²/hr was 18 in cows and 3 in man. The heat production was proportionately higher in cattle: at 80° F. the heat production in Cal/m²/hr was about 160 for superior lactating European cows, 100 for poorly lactating cows, 50 for lightly clothed man. Major cause of the lower heat tolerance of cattle is that their vaporization-increase (with increasing temperature) is S-shaped, which ceases to increase at about 80° F., with resulting rise in rectal temperature and decline in feed consumption, milk production, and thyroid activity; whereas man breaks out in sweat at 80° F. and evaporative cooling increases exponentially with increasing temperature.

P3. Growth comparisons for Holstein, Ayrshire, Guernsey, and Jersey males for the first six months of life. H. P. DAVIS, Univ. of Nebraska, Lincoln.

Twenty-yr. records of monthly body measurements of cattle at the Univ. of Nebraska furnished data for the following: body weights; heights—at withers, hooks, and pin bones; lengths—diagonal, body, and rump; widths at chest, hooks, and pin bones; depths—at chest and barrel; girths at barrel and chest; and hide thicknesses (double) at fore rib and at last rib. Numbers of animals varied somewhat but typically were as follows: Holsteins—birth 220, 6 mo. 104; Ayrshires—birth 58, 6 mo. 12; Guernseys—birth 25, 6 mo. 8; and Jerseys—birth 95, 6 mo. 31.

The weight averages in pounds were as follows: Holsteins—birth 100.4, 6 mo. 440.4, total gain 340.0, daily gain 1.86; Ayrshires—birth 81.2, 6 mo. 321.5, total gain 240.3, daily gain 1.32; Guernseys—birth 72.6, 6 mo. 329.1, total gain 266.5, daily gain 1.41; Jerseys—birth 57.5, 6 mo. 277.0, total gain 219.5, daily gain 1.20. In terms of birth weight as 1.00, the 6-mo. weights were: Holsteins 4.42; Ayrshires 3.96; Guernseys 4.54; and Jerseys 4.82. In most other comparisons the ratios to birth were closer together, which indicates that growth is much alike for each breed, with the Jerseys tending to grow a little faster and the Ayrshires a little slower than the others.

P4. The relationship of nutrition to reproductive performance of dairy cattle—a field study. W. A. HARDISON, S. L. KALISON, N. O. PRICE, R. W. ENGEL, and W. B. BELL, Virginia Agr. Expt. Sta., Blacksburg.

In July of 1953 the Virginia Agr. Expt. Sta. in cooperation with the Virginia Artificial Breeder's Assoc. initiated a field study to determine whether or not nutrition may be a factor in the inability of some dairy cows to conceive. Breeding records indicated lower conception rates in the Shenandoah Valley than in any other section of the state. Six cows were selected from each of 4 representative dairy farms in this section, and examining veterinarians indicated these cows were free of infectious disease conditions that might interfere with normal conception. Blood samples were taken at 3-mo. intervals for carotene, vitamin A, vitamin E, calcium, and phosphorus analyses. Additional information relative to breeding performance and feeding practices was obtained. Forage samples were taken for trace mineral analysis. One herd was dropped from the study after the 1st year because of poor management practices. To date there is no clear-cut evidence that cows in these herds are suffering from an insufficient intake of any of the nutrients mentioned above. There are indications that blood levels of carotene, vitamin A, and vitamin E are related to feeding and management. Some nonlegume forages in the Shenandoah Valley may be deficient in copper and cobalt.

P5. Factors affecting pregnancy interruption in artificial insemination of dairy cattle. T. Y. TANABE, C. E. HEIST, and J. O. ALMQUIST, The Pennsylvania State Univ., University Park.

One hundred five pregnant heifers and cows were inseminated just into the uterine body with 1 ml. of semen diluted with either heated milk or egg yolk–citrate with and without penicillin and streptomycin (1,000 u. of each per ml.). When antibiotics were added to the semen only 7.5% (4 of 53) of the pregnancies were interrupted as compared with 75.0% (39 of 52) interruptions when antibiotics were omitted. This difference is supported by the following bacteriological data: *(a)* 15 of the 16 uteri showing large numbers and heterogeneous types of bacteria involved semen without antibiotics, and 12 of the 15 showed pregnancy interruptions; *(b)* 19 of the 23 uteri showing few or no bacteria involved semen with antibiotics, and one of the 19 showed pregnancy interruption.

Although the incidence of interruptions did not vary greatly among the 4 stages of pregnancy (2, 3, 4, and 5 mo.), 34.9% (15 of 43) of the pregnancy interruptions were abortions which occurred primarily after inseminations at the 4th and 5th mo. of pregnancy. Antibiotics decreased the frequency of abortions (2 of 15).

In a second group of 24 animals one pregnancy interruption occurred after passage of an insemination tube devoid of semen into the uterine body. Few or no bacteria were found in 11 of 12 uteri maintaining normal pregnancy. Mid-cervical deposition of diluted semen without antibiotics in a third group of 13 animals interrupted pregnancy in one instance.

P6. Diverticula in the bovine oviducts. H. J. WEETH and P. R. TERNEN, Univ. of Nevada, Reno.

A previous study of bovine oviducts indicated epithelial-lined diverticula around the lumen

periphery. Because these diverticula may influence movements of spermatozoa and ova, a detailed study of their morphology has been completed. Serial sections were prepared from the ovarian, mid, and uterine region of tubes from 10 normal, mature heifers.

The diverticula are lined with the same type epithelium which covers the longitudinal folds of the tube. They originate in the lamina propria as solid nests of epithelial cells. Within 24μ a lumen develops in the pocket and eventually opens into the oviduct. Pockets open both anteriorly and posteriorly. The number, depth, and diameter of pockets increase from the uterine to the ovarian end. The average pocket depth was 57, 74, and 97μ in the low, mid, and upper regions, respectively, with a range of 24 to 348μ. Many diverticula have irregular lumina, especially toward the ovarian end of the tube. The diameters of many exceed those of ova. Lumina with minor axis diameters of more than 150μ were frequently observed in the mid and ovarian regions. These diverticula are large enough to hold spermatozoa and ova. Physiological studies of the diverticula are being undertaken.

P7. How do cow families develop? MOGENS PLUM and M. G. A. RUMERY, Univ. of Nebraska, Lincoln.

The Holstein herd at the Nebraska North Platte Expt. Sta. was founded in 1913 with 12 Holstein females. By the end of 1954 the herd consisted of 86 females, all descended from 4 of the original females. These 4 females were the daughters and granddaughters of 2 cows (designated 1 and 2) in the herd where they were purchased. The numbers of females in these 4 families were: 1A, 38; 1B, 29; 2A, 19; and 2B, 3. Thus, 74% descended from cow No. 1 and 26% from cow No. 2.

A study of the butterfat production of these 12 families shows that there were no significant differences in production levels. Family differences account for only 5% of the variance of the 1st year records, and 35% of the variance can be associated with the bulls used.

A comparison of the sex of offspring of cows in the 4 families having descendants in the herd today shows that the cows that have descendants today had 60% females and the cows that have no descendants now had only 42% females. Thus, the establishment of families depends upon the chance occurrence of a relatively large number of females in the family.

P8. The occurrence of estrus during pregnancy in several Holstein herds. H. R. DONOHO and H. E. RICKARD, The Ohio State Univ., Columbus.

A study was made in 5 large Holstein herds to determine the nature and occurrence of estrus in cows that had already conceived. The breeding dates were checked after the termination of all pregnancies that resulted in apparently normal calves. The calves were considered normal if they were average (or better) in size and wt. and presented no indications of any physical abnormality.

In 5 large herds, 1,454 calvings were studied. Approximately half of the cows were bred naturally and the others artificially. Analysis of the data indicated that *(a)* approximately 6% of the cows in the herds studied had one or more estrous cycles post-conception, *(b)* most cows which show estrus during pregnancy do so only a few times, *(c)* the average time for cows to show signs of post-conception estrus is approximately 78 days.

P9. Thyroidal influence on semen production and the behavior of bulls used in artificial breeding. W. S. GRIFFITH, CECIL BRANTON, H. C. KELLGREN, and G. F. D'ARENSBOURG, Louisiana Agr. Expt. Sta., Baton Rouge.

Eighteen bulls were studied over a 9-mo. period to determine what effects natural fluctuations in the level of thyroidal activity (as measured by plasma P.B.I.) exert upon semen production and service behavior.

Ejaculates collected during the period totaled 1,296 and were evaluated as to volume, % progressive motility, rate of movement, and concentration. Total spermatozoa, total motile spermatozoa, and % shippable ejaculates (initial concentration of 500×10^6 and progressive motility of 50%) were also calculated.

Measurements of the behavior included reaction time (interval elapsing between the time the bull is released and the time that he mounts the teaser animal), excitement score (based on a 14-point scale), thrust score (5-point scale), and % successful services (ejaculates produced divided by number of opportunities for ejaculation).

P10. Seasonal trends in plasma PBI levels, semen production, and fertility of bulls. CECIL BRANTON, W. S. GRIFFITH, T. E. PATRICK, J. E. JOHNSTON, and G. F. D'ARENSBOURG, Louisiana Agr. Expt. Sta., Baton Rouge.

Previous work has shown that semen quality and fertility of bulls in the Gulf Coast region declines markedly during the hot, humid months. Thyroidal activity is believed to be closely associated with these reactions. A study was begun in March, 1954, to determine the interrelationships of climatological, PBI, semen production, and fertility data.

Eighteen bulls (5 Jerseys, 5 Holsteins, 5 Guernseys, 1 Angus, 1 Hereford, and 1 Brahman) were studied. Semen collections were made at weekly intervals. All semen samples meeting minimum quality standards were used for artificial insemination. Plasma PBI was determined at monthly intervals. PBI levels were found to have a highly significant negative relationship to av. monthly maximum temperatures ($r = -0.251$). These values ranged from a high of 7.02γ% in the spring to a low of

3.26γ% in the summer. Fertility and % usable ejaculates appeared to have followed a similar trend.

P11. Semen production by a bull ejaculated three times per week for three consecutive years. N. L. VANDEMARK, L. J. BOYD, and F. N. BAKER, Univ. of Illinois, Urbana.

One Holstein bull from a study on ejaculation frequency has been collected 3 times per week for 3 consecutive years since puberty. This bull first showed sexual interest at 25 wk. of age and first ejaculated when 38 wk. old. During the 1st year, 153 ejaculates were produced that averaged 2.8 ml., with a sperm concentration of 1,089 × 10^6/ml and an av. initial motility of 56%. During the 2nd year, the average volume of 151 ejaculates increased to 3.8 ml., with a concentration of 1,530 × 10^6 sperm/ml and an average motility of 59%. During the 3rd year, 143 ejaculates averaged 5.0 ml. each with 1,513 × 10^6 sperm/ml and 57% motility.

Assuming that 10 × 10^6 sperm cells should be used for each insemination, this bull has produced a sufficient number of spermatozoa to breed a potential of 251,382 cows. During his 3rd year of semen production, this bull was proven fertile. There has been no indication of a reduction in libido or in semen quality.

P12. The effect of supplementary adenosine mono- and tri- phosphate on the metabolism of bovine spermatozoa.[1] F. J. GRUNFELD and C. P. MERILAN, Univ. of Missouri, Columbia.

Metabolic activity of bovine spermatozoa was studied with the Warburg respirometer, measuring the rate of respiration by O_2 uptake and the rate of anaerobic glycolysis by apparent CO_2 release in Krebs-Ringer phosphate or bicarbonate.

Additions of 2 levels of AMP, 30 mg.% and 60 mg.%, to whole semen showed no increase in the respiratory rate and had an inhibitory effect on anaerobic glycolysis, the inhibition increasing with increasing concentrations. Additions of 3 levels of AMP, 60, 120, 180 mg.%, to spermatozoa washed free of seminal fluid showed a definite increase in both rate of respiration and anaerobic glycolysis. The increase was proportional to concentration of the substrate. Additions of 0.006 *M*, 0.013 *M*, and 0.026 *M* ATP caused an increased rate of anaerobic glycolysis. The rate of increased activity was maintained for 15 to 30 min. and then returned to that of the controls.

Assays for ATP-ase activity were carried out with semen at varying times of storage. The micrograms of inorganic phosphorus released from ATP by 1 × 10^8 spermatozoa decreased directly with a decrease in motility.

[1] Contribution from the Missouri Agr. Expt. Sta., Journal Series No. 1515. Approved by the director.

P13. The effect of phosphate-containing and saline diluents on the aerobic metabolism of bull semen. G. W. SALISBURY and N. T. NAKABAYASHI, Univ. of Illinois, Urbana.

At the A.D.S.A. meetings in 1952 Bishop and Salisbury reported that phosphate-containing diluents depressed the respiratory activity of bull spermatozoa. These studies have been extended to include not only respiratory rate but also aerobic fructolysis. In 24 semen samples diluted 1:4 with 0.9% NaCl the mean rate of oxygen uptake/100 × 10^6 cells was 12.1μl. O_2 for the 1st hr. at 37° C., and averaged 11.6μl. O_2 per hr. for 4 hr. In an isotonic diluent composed of sodium dihydrogen and sodium monohydrogen phosphates adjusted to pH 7 the mean respiratory rate for the 1st hr. was 8.3μl/100 × 10^6 cells and averaged for 4 hr., 5.3μl/hr. The phosphate depressed both respiratory activity and livability of the cells as compared to the saline diluent. Fructose utilization in the saline was 74% of that in the phosphate, and lactic acid production was decreased even more. In saline diluents of varying osmotic pressure the highest respiration and aerobic fructolysis at 37° C. resulted with solutions isoosmotic and hyperosmotic (up to +50%) to seminal plasma. With phosphate diluent, optimum respiration and aerobic fructolysis occurred in hypoosmotic solutions, and each increase in the concentration of phosphate reduced both respiration and aerobic fructolysis.

P14. Influence of dilution rate on metabolism of semen in phosphate buffer and in milk diluter. M. H. EHLERS and R. E. ERB, State College of Washington, Pullman.

Fructose and lactic acid were measured in bull semen diluted with M/15 phosphate buffer, and lactic acid in semen diluted with heated homogenized whole milk. Measurements were before and after 1 hr. incubation at 37° C. With 1 group of samples, comparisons were with 0, 1, 2, 3, 4, 6, 8, 16, and 24 parts phosphate. Sperm count in these samples averaged 1.247 billion/ml and fructose 5.49 mg/ml semen. At 1 part semen to 4 parts phosphate (standard dilution) fructose utilization averaged 2.02 mg. and lactic acid accumulation 1.32 mg/billion sperm. With 0 parts buffer, fructose utilization was 73% and lactic acid accumulation 68% of the amount recorded for the standard. In the range of 1 through 8 parts phosphate there was no pronounced influence of dilution. At the higher dilutions fructolysis was markedly lower. With 24 parts phosphate, fructose utilization was 47% and lactic acid accumulation 38% of that observed for the standard. Other samples were diluted with 4, 8, 16, and 24 parts milk, and these were compared with a portion diluted at the rate of 1 part semen to 4 parts phosphate. Sperm count averaged 1.405 billion/ml and fructose 5.55 mg/ml semen. Fructose utilization was 2.28

mg. and lactic acid accumulation 1.83 mg/billion sperm for the standard. In 4 parts milk lactic acid accumulation averaged 2.06 mg/billion sperm. There was no decline in lactic acid accumulation with increased dilution in milk; in all samples lactic acid formed was not greater than could be accounted for by the seminal fructose initially present.

P15. Study of possible factors related to freezability of spermatozoa. J. I. OHMS and E. L. WILLETT, American Fdn. for the Study of Genetics, Madison, Wis.

Three possible factors related to freezability of spermatozoa were studied: seminal plasma, maturation, and specific gravity. The 1st experiment involved 10 collections from 7 bulls. Spermatozoa from 1st and 2nd ejaculates were subjected to 5 treatments: *(a)* uncentrifuged; *(b)* centrifuged twice and resuspended in supernatant; *(c)* washed twice by centrifuging in yolk–citrate and resuspended in yolk–citrate plus plasma from the same ejaculate; *(d)* same as *c* but with plasma from other ejaculate; and *(e)* same as *c* but resuspended in yolk–citrate only. There were no significant differences in progressive motility after freezing and thawing among treatments within ejaculates, but the mean for the 2nd ejaculate (33.0) was highly significantly greater than for the 1st (17.6). In another experiment spermatozoa were obtained from ampullae, the small tubes of the ductus deferens, and the cauda and caput epididymides of each of 15 slaughterhouse bulls. Very few motile spermatozoa were observed from the caput. After freezing and thawing, there was no significant difference in motility of spermatozoa from ampullae and cauda. In a 3rd experiment (8 collections) the difference between the mean specific gravity of spermatozoa from 1st ejaculates (1.231) and 3rd ejaculates (1.210) was highly significant.

P16. The use of mechanical refrigeration for storage of frozen semen. W. M. ETGEN and T. M. LUDWICK, The Ohio State Univ., Columbus.

Twelve ejaculates from 9 bulls were used in a split sample study to determine the comparative value of mechanical and Dry Ice refrigeration for storing frozen semen. Eighty 1-ml. ampules from each ejaculate were frozen and divided into 2 groups. One group was placed under Dry Ice refrigeration at approximately –72° C. The other was kept in a mechanical refrigerator at –96° C. Forty ampules from each ejaculate were used to study the effects of constant storage under the 2 conditions. The remaining samples from each ejaculate in each respective box were transferred to the opposite storage condition at 3 and 12 wk.

Two ampules from every ejaculate under each storage condition were evaluated at specified intervals for per cent of live cells and motility. These microscopic estimates of quality were recorded and evaluated with the following trends: *(a)* Frozen semen stored under mechanical refrigeration at –96° C. exhibited significantly higher survival rates than semen stored under Dry Ice conditions; *(b)* the transfer of frozen semen samples to the opposite condition, after periods of storage at one temperature, tends to reduce quality somewhat. The reduction is probably not below that of semen stored at the Dry Ice temperature.

P17. Electroejaculation in the bull. H. J. HILL, F. S. SCOTT, NORMAN HOMAN, and F. X. GASSNER, Colorado A & M College, Fort Collins.

A practical method of electroejaculation of the bull with a new electronic ejaculator is discussed. (Marden, *J. Dairy Sci.*, **37**: 556. 1954.) Semen samples have been taken from 400 beef bulls and 10 dairy bulls over the past 10 mo. One to several ejaculates were collected, depending on the final evaluation assigned each successive sample. Information on 100 bulls representing 76 Hereford, 13 Angus, 6 Jersey, 3 Milking Shorthorn, and 2 Brown Swiss has been selected at random from over 400 case records.

Evaluations of each of 6 physical criteria [vol., conc., % motile, % alive (Blom), morphology, and pH] were averaged within age groups (e.g., 8-11 mo.; 12 mo. to 2 yr.; 2½ to 5 yr.; over 5 yr.). There was no indication that electrically ejaculated semen samples differ from naturally ejaculated samples collected with the artificial vagina. No adverse physical effects were noted. Studies on 4 bulls subjected to ejaculatory exhaustion by electro means show that up to 35 fractions can be taken from a bull within 75 min. without adverse effect. Preliminary data indicate that there is no appreciable difference between nonreturn rates.

P18. Effect of concentrate level on in vitro incorporation of S-35-labeled sulfate by rumen microbiota. R. S. EMERY, C. K. SMITH, R. L. SALSBURY, and C. F. HUFFMAN, Michigan State College, East Lansing.

Fresh rumen liquid from 4 fistulated bovines (receiving either hay alone or 75% of their TDN as concentrate) was incubated anaerobically with added substrate and isotopic inorganic sulfate as tracer. Alfalfa meal substrate was used with inocula from hay-fed donors; grain with inocula from concentrate-fed donors.

Relative sulfate activity incorporated into cyst(e)ine, methionine, and glutathione sulfur was determined at 0.5-hr. intervals through 3 hr. Sulfate incorporation by the grain microbiota was significantly ($P < 2\%$) greater than that by the hay microbiota. This is largely due to a significantly greater ($P < 5\%$) portion of the total "sulfate pool" (sulfur in equilibrium with the tracer sulfate) being available for incorporation. Relative activities found in cyst(e)ine and methionine were roughly equal re-

gardless of substrate and accounted for most of the incorporation, although glutathione contained appreciable activity. Microradioautographs suggest that only a few of the many species in the rumen are responsible for this incorporation.

The data reasonably fit an exponential equation in which the incorporation rate is less than 5% per min. of that sulfate available for uptake at any given time. The rate with grain appears to be slightly higher than with hay.

P19. Studies on the dissimilation of purines and pyrimidines by bovine rumen bacteria. P. JURTSHUK, JR., and F. G. HUETER, Univ. of Maryland, College Park.

Since adenine, guanine, xanthine, hypoxanthine, and possibly thymine and uracil are present in grassland herbage, it was considered of interest to determine their utilization by bovine rumen bacteria.

Preliminary work indicates that when these compounds are tested by the mixed suspension technique some dissimilation occurs. Values obtained for the % ammonia released were as follows: adenine sulfate, 0%; guanine hydrochloride, 11%; hypoxanthine, 2.8%; xanthine, 7%; urie acid, 6%; uracil, 9%; and thymine, 11%.

P20. Further studies on the influence of diet on the development of the ruminant stomach. R. G. WARNER, C. H. GRIPPIN, W. P. FLATT, and J. K. LOOSLI, Cornell Univ., Ithaca, N. Y.

Twenty-four 3-day-old Holstein bulls were allotted to one of the following treatments: (I) mineralized milk; (II) 350 lb. milk plus dry calf starter; (III) 350 lb. milk plus hay. Two calves from each group were slaughtered at 4, 7, 10, and 13 wk. Reticulo-rumen volumes (l/45.4 kg. of empty body wt.) were determined and at 4 and 13 wk. were, respectively: Grp. I, 3.7, 3.2; Grp. II, 5.8, 12.5; Grp. III, 4.4, 31.4. The omasum followed a similar trend. Reticulo-rumen and omasal tissue (g/45.4 kg. empty body wt.) were, respectively: Grp. I (4 wk.) 34.4, 12.4, (13 wk.) 38.4, 10.0; Grp. II (4 wk.) 53.3, 9.7, (13 wk.) 146.2, 22.9; Grp. III (4 wk.) 53.2, 8.9, (13 wk.) 165.8, 25.9. These data suggest that dry feed stimulates the tissue growth but hay per se stretches these organs. Papillary development of the rumen wall of Grp. II was as great as that of Grp. III but rudimentary in Grp. I. Indications are that the development of the rumen lining may be stimulated by chemical entities in the ingesta. If a rough material is required, dry calf starter contains an adequate amount. Calves receiving nylon bristles as the sole dry feed showed no papillary development. Gross abomasal development was not altered by diet.

P21. Lactic acid production in the rumen. D. R. WALDO and L. H. SCHULTZ, Cornell Univ., Ithaca, N. Y.

Production and disappearance of lactic acid in the rumen of dairy steers were measured by determining levels of lactic acid in rumen liquor before and at short intervals after feeding.

The effect of roughage type, with roughage as the only feed, was investigated in a 4 × 4 design. Lactic acid levels were generally below 1 mg/100 ml. of rumen liquor before feeding, rose after feeding, and dropped to prefeeding levels by 4 hr. Peak concentrations found in the rumen, expressed in mg/100 ml. of rumen liquor were 22 at 1 hr. on grass hay, 20 at 1 hr. on alfalfa hay, 142 at ½ hr. on corn silage, and 209 at ½ hr. on hay crop silage.

With the same design and steers, grain was added to a grass-hay ration at 0, 2½, 5, and 7½ lb/day, with twice-a-day feeding. The grain increased lactic acid production over the hay alone, but not consistently in proportion to the level of grain feeding. The peak concentration of lactic acid in mg/100 ml. of rumen liquor on hay alone was 41 at 1 hr., whereas on the hay plus 7½ lb. of grain it was 58 at ½ hr.

Cellulose, starch, and pyruvic acid were administered singly to 2 fistulated animals with no rise in lactic acid. One lb. of glucose produced peaks of 148 and 127 mg/100 ml. at 1 hr. and ½ hr., respectively. Administered lactic acid disappeared rapidly from the rumen.

P22. Relation between age of calf, blood glucose, blood and rumen levels of volatile fatty acids, and in vitro cellulose digestion. R. D. MCCARTHY and E. M. KESLER, Pennsylvania Agr. Expt. Sta., University Park.

Blood and rumen juice samples were collected from 6 male Holstein calves at 7-day intervals until 105 days of age. Calves received 5 lb. of whole milk twice daily until the 42nd day plus starter to a maximum of 5 lb. and alfalfa hay ad lib. Blood was drawn from the jugular vein and rumen juice was obtained by stomach pump. Blood glucose and reducing substances in the rumen juice were determined by the Somogyi method. Total volatile fatty acids were obtained by steam distillation. Cellulose digesting power of rumen juice was measured in the artificial rumen.

Blood glucose decreased from 113.4 mg/100 ml. at 7 days to 43.9 mg. at 42 days and then remained in the 40-60 mg. range. Volatile fatty acids in the blood increased from 2.28 mg/100 ml. at 7 days to 6.24 mg. at 105 days. Reducing substances in rumen juice fluctuated between 3-6 mg/100 ml. throughout the trial. Volatile fatty acids in rumen juice increased from 156 mg/100 ml. at 7 days to 794 mg. at 63 days, thereafter leveling off around 600 mg. Cellulose digestion increased steadily from 48.6% at 7 days to 87.1% at 105 days.

P23. The effect of protein level on rumen volatile fatty acids. N. S. WOODHOUSE, R. F. DAVIS, and G. H. BECK, Univ. of Maryland, College Park.

Reports by El-Shazly and Annison have suggested that dietary protein may affect the pro-

duction of fatty acids in the rumen, particularly branched chain acids and isomers of butyrate and valerate. Experiments designed to test a possible protein effect were undertaken with 9 lactating cows assigned to 3 replicates of a 3 × 3 Latin square. A basal ration of timothy hay, corn silage, and corn and cob meal was fed. Experimental variables consisted of the replacement of the corn with 2 levels of soybean oil meal. These treatments provided approximately 0.19, 0.36, and 0.54 lb. of total protein per 100 lb. of body wt. Samples of rumen contents were obtained orally on 3 consecutive days after 14 and 21 days on each treatment and subjected to chromatographic analysis for acetic, propionic, butyric, valeric, and "higher" acids. Statistically significant differences were obtained as follows. Total fatty acids present and total amounts of propionic, butyric, and valeric acids were increased on the high protein ration. The percentage of acetate was lowered and that of butyrate and valerate raised on the high protein ration. Differences between samples taken after 2-wk. or 3-wk. preliminary periods were not statistically significant.

P24. Studies on the bloat syndrome. J. T. Blake, N. L. Jacobson, and R. S. Allen, Iowa State College, Ames.

Acute bloat was induced by oral administration of juices expelled from freshly cut alfalfa. Mild bloat was induced with concentrated and with spray-dried alfalfa juice.[1] The diet most effective in producing feed-lot type bloat was composed of ground corn, 60%; soybean oil meal, 17%; alfalfa meal, 20%; and minerals, 3%. Incidence of the latter type of bloat was not greatly reduced by *(a)* substitution of dextrose for 1/3 of the corn, *(b)* substitution of dehydrated white clover or dehydrated cereal grasses for the alfalfa, or *(c)* removal of the alfalfa meal. Several cases of chronic feed-lot type bloat were observed among animals receiving a diet containing at least 30% coarse hay.

Rumen fluid from bloated animals receiving the dry feeds was more foamy and the surface tension was higher than that of fluid from the same animals when not bloated. Changes were less distinct in the animals bloated with alfalfa juice.

In vitro surface tension values for rumen fluid increased as temperature was reduced from 37° to 20° C. but remained constant when temperature was maintained at 37°. Marked decreases in surface tension in vitro, and lesser changes in vivo, were induced by addition of turpentine, a detergent, and various anti-frothing agents.

[1] Concentrated alfalfa juice and dried alfalfa juice supplied by Western Condensing Co., Petaluma, Calif., through the courtesy of P. D. Clary, Jr.

P25. The effect of alfalfa saponin on rumen activity in sheep. H. W. Colvin, Jr., P. T. Cupps, and C. R. Thompson,[1] Univ. of California, Davis.

In sheep on diets of either Sudan grass or alfalfa tops, 100 g. of alfalfa saponin dissolved in 1 l. of 5% glucose solution caused death within 1½ to 2½ hr. One hundred g. of saponin from the same lot taken up in 1 l. 0.9% NaCl solution produced death in 2¼ hr. in a sheep fasted 48 hr. After the administration of the saponin solutions, rumen motility ceased almost immediately. Foamy bloat was observed when the glucose and saponin were introduced into the rumen together. Postmortem findings indicated that death was associated with an intense submucosal and subserosal hemorrhagic condition of the gastro-intestinal tract.

In other trials, rumen motility was markedly depressed by either 25 g. of alfalfa tops or 12.5 g. of the alfalfa saponin. During the experiments in which the saponin was used, oat hay was the basal diet. When alfalfa tops were fed, the animal became slightly bloated; however, no distention was observed following the use of alfalfa saponin in the animals on an oat hay diet.

[1] Western Utilization Research Branch, USDA.

P26. Ingesta volume increase in rumen contents of cattle on normal and bloat-producing rations. Don Jacobson, Univ. of Maryland, College Park, and Ivan Lindahl, Agricultural Research Center, Beltsville, Md.

In cattle on bloat-producing rations it was observed that the rumen contents after removal increased in volume with time. This increase is apparently due to the trapping of microamounts of gas. In order to measure the ingesta volume increase (IVI) an aliquot (100 ml.) of freshly drawn rumen contents in a 250-ml. graduated cylinder was placed in a constant temperature bath (39° C.) and the increase in volume recorded at 1 hr. in per cent. The average IVI of 13 samples from 6 animals on a low-hay, high-concentrate, bloat-producing ration was 89, whereas when these animals were fed hay and grain in a 2 to 1 ratio the average IVI was reduced to 67. When tested by the *t* test, the IVI values on the two rations were significantly different at the 1% level. A sudden change to a high-hay ration did not reduce bloat appreciably the first 4 days, but after this time there was a gradual reduction. On the low-hay ration the rumen contents were homogenous, whereas after changing to a high-hay ration there was a gradual development of a liquid phase which separated from the less dense material.

P27. Volatile fatty acids and pH in rumen contents of cud-inoculated and uninoculated calves fed high roughage pellets. H. R. Conrad, J. W. Hibbs, J. H. Vandersall, and W. D. Pounden, Ohio Agr. Expt. Sta., Wooster.

Thirty Jersey calves, half of which were inoculated, were used to determine the effect of fresh cud inoculations on the changes in the volatile fatty acid content and pH of rumen juice during the first 16 wk. Ground alfalfa hay (½-in. mesh) and a simple grain mixture in a 2:1 ratio were pelleted and fed free choice as the only dry feed. Limited whole milk was fed to 7 wk. of age. No significant differences in the rumen level of steam volatile acids was observed between cud-inoculated and uninoculated calves. Chromatographic separation of the volatile fatty acids in the rumen juice at 6, 8, 12, and 16 wk. of age showed no consistent differences between inoculated and uninoculated calves in the amount of acetic acid. However, the uninoculated calves had a consistently higher level of propionic acid and a lower level of butyric acid. The molar percentage and the grams per liter were, respectively: acetic, 63.2 and 3.79; propionic, 19.5 and 1.44; and butyric, 17.3 and 1.57; for the cud inoculated calves, compared to: acetic, 61.9 and 3.72; propionic, 25.1 and 1.84; and butyric 13.0 and 1.14 for the uninoculated calves. The inoculated calves tended to be somewhat lower in rumen pH from 2-14 wk. Additional evidence that these differences in volatile fatty acids and rumen pH were associated with the kinds of rumen microorganisms established was obtained in concurrent studies of cellulose and protein digestion and in vitro studies.

P28. Methods of feeding and rumen inoculation as they affect the growth and development of young dairy calves. R. A. Ackerman and J. E. Fike, West Virginia Univ., Morgantown.

Three groups of 18 calves were fed from birth to 16 wk. of age as follows: Grp. 1, calf starter and mixed hay at will; Grp. 2, calf starter at will and limited mixed hay; and Grp. 3, limited calf starter and mixed hay at will. The starter was limited to 4 lb/day for each group.

The first 2 groups grew at about the same rate and cost per lb. of gain was about equal, whereas the third group gained only about ¾ as much as the others and made the gain at a slightly higher cost per lb.

Half of the calves in each group were inoculated with fresh cud from a healthy cow each wk. from birth until 6 wk. of age. The inoculation showed no advantage under any feeding system. Inoculated calves showed protozoa at all times after their first inoculation. Although reasonable care was taken to prevent natural inoculation of the noninoculated calves, rumen samples of these calves usually showed similar microorganisms, including protozoa, by the time the calf was eating an appreciable amount of hay. Under our regular management conditions, calves apparently become inoculated naturally at an early age.

P29. Further studies on an iodine staining substance produced by bovine rumen bacteria. R. J. Gibbons and R. N. Doetsch, Univ. of Maryland, College Park.

A method has been devised for quantitatively estimating the amount of an iodine staining substance (I.S.S.) produced from carbohydrates by washed suspensions of bovine rumen bacteria. With this method, the effect of urea, cobalt, inorganic salt mixtures, and a chelating agent on the formation of I.S.S. has been studied.

The I.S.S. polysaccharide has been extracted from bovine rumen bacteria in crude form. The extracts have been subjected to acid hydrolysis, and the constituent monosaccharides have been identified by means of paper chromatographic methods.

Cell-free extracts of rumen bacteria have been found capable of hydrolyzing the polysaccharide, and the constituents of this enzymatic degradation also have been identified.

P30. The development of the flora and fauna in the rumen of growing calves. M. P. Bryant, Nola Small, and L. A. Burkey, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Samples of rumen contents from 1 Holstein and 2 Jersey calves were studied with direct microscopic and cultural methods. The calves were fed whole milk for 60 days and alfalfa hay and a simple grain mixture (15.4% protein) after 10 days of age. A definite sequence in the establishment of different kinds of protozoa was found. Small flagellates were present at 1 wk.; entodinia, 17-20 wk.; diplodinia, 27-28 wk.; and holotrichs, 33-37 wk. after birth. The predominating bacteria in calves 1-3 wk. old were different from those of mature animals. They were gradually displaced by organisms typical of the mature animal, and at 9 wk. of age the predominating bacteria were chiefly kinds found in mature animals. In week-old calves cellulolytic bacteria were low in numbers, and facultative anaerobes, streptococci, coliforms, and lactate-fermenting bacteria were high in numbers as compared to 9 to 13-week-old calves and mature cows. Under the relatively isolated condition of the nutrition herd calves at Beltsville, many of the kinds of bacteria typical of mature animals became established in very young calves. The establishment of the full complement of protozoa was delayed until the calves were older and in direct contact with mature animals.

P31. Observations on the use of mixed suspensions of bovine rumen bacteria as a technique of the rumen microbiologist. R. N. Doetsch, J. C. Shaw, J. J. McNeill, and P. Jurtshuk, Jr., Univ. of Maryland, College Park.

Mixed suspensions of bovine rumen bacteria are prepared by differentially centrifuging them from freshly drawn rumen liquor. The cells are considered essentially as a tissue and are studied by conventional manometric techniques. The effect of various physical and chemical conditions on this nonproliferating, enzymatically competing microbial population may easily be investigated.

Among the advantages of this technique are: (1) there is little or no activity in the absence of added substrate, (2) the reactions may be measured over a convenient period of time, (3) the end-products may be determined, (4) the microbial flora has not been cultured artificially. The main disadvantages are: (1) the rumen protozoa are largely omitted, and (2) the reactions of any given "species" of rumen microorganism are not obtained.

The technique has been confirmed in the few in vivo studies made thus far. It is suggested that some abnormalities in rumen physiology might be referred to rumen microorganisms if in vitro reactions from abnormal animals are at variance. Expensive, time-consuming in vivo work might largely be eliminated by using in vitro reactions as a guide. Finally, basic information needed for supplying the necessary framework for further experimentation may be provided by this technique, and it is expected that it will be of use to the rumen microbiologist as well as to the animal physiologist.

P32. Studies on the methane and hydrogen metabolism of bovine rumen bacteria. J. J. McNeill and D. R. Jacobson, Univ. of Maryland, College Park.

Methane is produced in a hydrogen atmosphere when a source of carbon dioxide is supplied to mixed suspensions of bovine rumen bacteria. Evidence has been uncovered which suggests that failure to find more than trace amounts of hydrogen in the rumen can be ascribed to the presence of extremely active hydrogenase systems in the rumen bacteria suspensions. It has been impossible thus far to demonstrate hydrogen production by rumen bacteria even when using such a potentially hydrogen-rich source as formate. Hydrogen was sought for in Dixon-Keilin flasks with palladium black as a hydrogen absorbent.

In vitro experiments in a hydrogen atmosphere show almost complete methane production from various carbon substrates tested; carbon dioxide cannot be demonstrated under these conditions. This suggests that available hydrogen is the limiting factor in methane production in the normal rumen. Under experimental conditions, sufficient hydrogen and hydrogenase are present to reduce all of the carbon dioxide produced to methane.

P33. The rates of cellulose digestion in vitro using different sources of cellulose. R. L. Salsbury, C. K. Smith, and C. F. Huffman, Michigan Agr. Expt. Sta., East Lansing.

The cellulose digested in vitro during incubation periods of 3 to 24 hr. was determined for different roughages, for a wood cellulose, and for cotton linters. The miniature artificial rumen was used for incubation, and cellulose was determined by the method of Crampton and Maynard, with some modifications. A greater percentage cellulose digestion was obtained in the 24-hr. incubation period with wood cellulose than with the other substrates, but alfalfa leaf meal showed the highest initial rate of cellulose digestion. When the percentage cellulose digestion (as percentage of the 24-hr. digestion) obtained for different incubation periods was plotted on a logarithmic scale, it was found that timothy hay and alfalfa leaf meal gave smooth curves showing a uniform decrease in rate. The curves obtained for cotton linters and for the wood cellulose showed a characteristic retardation after an initially high rate of digestion.

P34. Cellulolytic activity of bovine rumen liquid upon a soluble cellulose derivative. J. L. Cason and W. E. Thomas, North Carolina State College, Raleigh.

Rumen liquid was collected from a fistulated cow, strained through 4 thicknesses of cheese cloth, centrifuged to remove large particles of ingesta and protozoa, filtered through filter paper of medium porosity, and stored under toluene at 4° C. until used. A water-soluble cellulose derivative, CMC-70-M (carboxymethyl cellulose—substitution No. 70—medium viscosity) made up in a sodium hydroxide-citrate buffer (pH 4.9 to 5.0) was used. One ml. of rumen liquid and 5 ml. of CMC-70-M solution were placed in an Ostwald-Fenske viscometer, giving a final concentration of 1% CMC-70-M. The viscosity was determined at specified intervals and the % hydrolysis of the CMC-70-M calculated with 50% hydrolysis taken as the end point. The cellulolytic factor in rumen liquid was found to be heat-labile, stable to storage at 4° C. under toluene, and stable to dilution with boiled rumen liquid. Liquid collected approximately 6 hr. after feeding was 9 times as active as that collected after 24 hr. The cellulolytic activity was influenced by changes in the ration. Valeric acid or ferrous sulfate at high concentrations had an inhibitory effect upon the action of the cellulolytic factor present in rumen liquid.

P35. Experimental udder growth and lactation in infertile dairy heifers.[1] H. YAMAMOTO and C. W. TURNER, Univ. of Missouri, Columbia.

Research with experimental animals indicates that optimal growth of the lobule-alveolar system is obtained by the injection of progesterone and estrogen at ratios of about 1,000 to 1. Since 100 mg. progesterone daily will maintain pregnancy in heifers from which the corpora lutea have been removed, the level of 100 mg. progesterone to 100γ estrogen was injected daily into 5 sterile heifers for 180 days, which is comparable to the duration of growth of the udder during normal pregnancy. At 6 mo. the lactation phase was initiated with daily injections of 3.0 mg. estrogen for 14 days. Twice daily milking was commenced a day after initial estrogen stimulation. A marked increase in milk secretion began after 3 to 6 days in 4 Jerseys and after 15 days in 1 Holstein. Although the hormone treatment stimulated hyperemia of the udder, marked enlargement of the udder occurred only after the lactation phase was initiated. To date, 3 heifers have reached maximum production after 8 to 9 wk. of lactation. The av. daily production was 18, 23, and 30 lb., respectively. The comparison of the av. maximum daily yields of milk with the records of fraternal and maternal half-sisters of 2 of the heifers and with the twin of the third indicates that they produced 66%, 137%, and 106%, respectively.

[1] Contribution from the Dept. of Dairy Husbandry, Mo. Agr. Expt. Sta., Journal Series No. 1519. Approved by the director.

P36. Mammary gland development in heifer calves. R. P. REECE, New Jersey Agr. Expt. Sta., New Brunswick.

Fourteen sexually immature heifer calves were used in an attempt to induce precocious development of the duct system of the mammary gland. Estrogen alone, either by inunction or subcutaneous administration, was ineffective in stimulating growth of the mammary duct system although teat growth occurred. Estrogen in combination with other hormones appeared to stimulate the development of the gland cistern region.

P37. The effects of the hormones progesterone and estrogen in initiating lactation in dairy cows. RALPH WILLIAMS, O. A. CHILDS, and DAN SMITH, Southern State College, and C. W. TURNER, Univ. of Missouri, Columbia.

Of the 8 Jerseys injected daily with progesterone and estradiol for 5½ mo. followed by an increased estradiol injection for 10 days, 7 produced daily from 16 to 29 lb. of milk. One group of 4 animals received 100 mg. of progesterone and 100γ of estradiol daily and the other 4 received 100 mg. of progesterone and 33γ of estradiol. All animals were injected with 6 mg. of estradiol for 10 days after milking was started. One cow developed a pelvic disorder during the increased injection of estradiol. No difference in production has been noted as a result of different ratios of progesterone and estradiol.

The remaining 6 cows showed normal estrous cycles after having 3 to 4 irregular cycles. One cow conceived with one service. The remainder have not conceived after 7 and 8 services. The 6 cows are producing from 7 to 18 lb. of milk daily per cow 6 mo. after the initiation of lactation with hormones.

P38. Milk production of first-calf heifers following prepartal administration of growth hormone. A. C. CHUNG, Univ. of Maryland, College Park.

Two sets of identical twin heifers and 1 set of identical twin older cows were used to observe the effect of growth hormone (GH) administered prepartum or prepartum and early postpartum. One twin heifer, which received 100 mg. GH intramuscularly daily for 9 days prepartum and 16 days postpartum, produced 6,331 lb. of FCM in 280 days, whereas the identical twin control produced 5,122 lb. A second heifer, which received 100 mg. GH daily for 23 days prepartum, has produced 3,110 lb. of FCM in 120 days, whereas its identical twin control has produced 2,584 lb. Feed efficiency was increased 20% in both cases. GH given postpartum to older cows was shown to maintain increased milk production for 7 wk., with a rapid return to normal after injections were discontinued. In an older set of identical twins, GH prepartum did not result in a sustained milk production. A comparison of the effects of GH and the equivalent amount of TSH on 6 cows in a reversal experiment of 2 injection periods of 7 and 12 days demonstrated conclusively that the galactopoetic effect of GH is not due to the TSH existing in the GH as a contaminant.

P39. The effect of various hormones upon udder development and milk secretion in dairy cattle. H. L. DALTON, Univ. of Georgia, Athens.

One animal from each of 2 sets of identical twins, 4 and 8 mo. of age, was injected with stilbestrol and progesterone to obtain superficial udder growth. The experimental animals had a greater wt. gain and growth of udder tissue than the controls. At parturition in the older set, the udder growth gain had been lost. To date, production is equal. The experimental animal of the younger set would not conceive. Four animals had implants of stilbestrol and progesterone pellets, and 3 animals responded satisfactorily. All 4 animals were injected for 9 wk. with stilbestrol; the animal not responding previously failed to secrete. After 60 days lactation, thyroprotein was fed for 90 days. These animals were more persistent than con-

trols except for the week thyroprotein was withdrawn. Five animals received daily injections of *(a)* stilbestrol and progesterone and *(b)* stilbestrol on alternate weeks until production leveled off. Two animals had normal secretion after 11 days, 2 after 15 days, and 1 animal failed to respond. Of the methods used, this appears to be more satisfactory. One of a set of twins received growth hormone 6 days prepartum and 15 days postpartum. After 75 days, production was continuing at a higher level than that of her mate.

P40. The association of lactogenic hormone labeled with radioactive iodine with the cytoplasmic nucleoprotein of the mammary gland cell.[1] W. F. WILLIAMS and C. W. TURNER, Univ. of Missouri, Columbia.

A previous study (Williams and Turner, *Proc. Soc. Exptl. Biol. Med.*, **85**: 524) has shown that the majority of labeled lactogenic hormone which enters the pseudopregnant rabbit mammary gland cell becomes associated with the particulate bodies of the cell, the mitochondria and microsomes. Since these portions of the cell are active in the metabolism of the cell, a study was made to determine the nature of the association.

It has been found that more than 95% of the radioactivity can be precipitated with 5% trichloroacetic acid, indicating that the radioactive iodine is attached to large molecules in the cell. Two series of experiments have been carried out with procedures that specifically precipitate nucleoproteins—precipitation with 4 *M* guanidine hydrochloride and with *M*/200 streptomycin. It was found that the 4 *M* guanidine hydrochloride precipitated 74.4 ± 14.2% of the radioactivity and the *M*/200 streptomycin precipitated 85.6 ± 10.3%. These precipitates give positive responses for the purine bases and for pentose sugar and positive Biuret and Xanthoproteic tests.

This work indicates that a "lactogenic hormone–nucleoprotein complex" of some sort is formed in the mammary gland cell. This may be important to the function of the lactogenic hormone in view of the probable role of the nucleic acids and nucleoproteins in energy transfer and protein synthesis.

[1] Contribution from the Dept. of Dairy Husbandry, Mo. Agr. Expt. Sta., Journal Series No. 1518. Approved by the director.

P41. Effects of cortisone, hydrocortisone, and ACTH on mammary growth and pituitary prolactin content of rats. R. M. JOHNSON and JOSEPH MEITES, Michigan State College, East Lansing.

Forty-two mature female rats were divided into 4 groups of 10 or 12 each and were subcutaneously injected as follows for 10 days: (1) controls, saline only; (2) 2 mg. cortisone daily; (3) 2 mg. hydrocortisone daily; (4) 2 mg. ACTH (Armour's) daily. On the 11th day the rats were killed and their pituitaries were removed for prolactin assay with pigeons. The right inguinal mammary gland also was removed from each rat, fixed in Bouins fluid over night, and stained with Delafield's hematoxylin.

Cortisone, and particularly hydrocortisone, reduced body wt., but ACTH had no effect. Gross mounts of the mammary glands of the control rats showed the presence of only bare ducts with few to a moderate number of buds on the ducts. Cortisone and hydrocortisone induced marked branching and lobule-alveolar growth. ACTH was least effective in increasing mammary growth at the dosage level employed. All three hormones increased the prolactin content of the pituitary (ACTH about 100% and cortisone and hydrocortisone about 40 to 50% each). Although further work with these hormones will be required, this experiment suggests that the glucocorticoids may have a role in the normal development of the mammary gland and initiation of lactation.

P42. Estimation of the L-thyroxine secretion rate of dairy animals.[1] G. W. PIPES and H. RUPPERT, Univ. of Missouri, Columbia.

Studies have been made of the minimum daily dose of L-thyroxine necessary to inhibit thyroid function in goats, dairy heifers, and mature dairy cows as indicated by radioiodine. I^{131} has been administered at the rate of 70μc. per 100 lb. body weight. After the maximum concentration of I^{131} is reached in the thyroid gland, L-thyroxine is administered at levels above the daily secretion rate. Thyroid function is inhibited via the pituitary, radio thyroxine disappears from the blood, and the release of radioactive compounds from the thyroid is inhibited. The daily dose of L-thyroxine is reduced progressively until thyroid activity is resumed. At this time the radioactivity of the blood increases, and loss of radioiodine from the thyroid becomes apparent.

With this technique it has been found that a single dose of 0.5 mg. of L-thyroxine per 100 lb. body wt. inhibits thyroid activity for 3 to 4 days in mature animals. Daily doses of 0.075 mg. of L-thyroxine per 100 lb. body wt. prevent thyroid activity in goats, but at the 0.05 mg. level thyroid activity becomes apparent.

The thyroid secretion rate of cows and heifers has been found to range between 0.08 and 0.02 mg. of L-thyroxine per 100 lb. body wt. per day.

[1] Contribution from the Department of Dairy Husbandry, Mo. Agr. Expt. Sta., Journal Series No. 1517. Approved by the director.

P43. Thyroxine and iodine determination in the bovine using a paper chromatography-photoelectric colorimetry technique. J. F. LONG, J. W. HIBBS, and L. O. GILMORE, Ohio Agr. Expt. Sta., Wooster.

Although the serum PBI test is a useful means of evaluating the thyroid hormone level

under usual conditions of iodine intake, it cannot be used where iodine intake is high, because of the formation of noncalorigenic iodinated serum proteins. Thus, many circumstances (high iodine feeding, thyroprotein feeding, and iodine medication) prevent satisfactory chemical evaluation of the hormone level. To overcome this difficulty a method has been developed with which highly iodinated serum, milk, and urine samples can be satisfactorily analyzed. The thyroxine fraction is separated from the remaining iodine-containing compounds by differential rates of migration on chromatographic paper. The paper is then cut into 1-in. sections and placed in colorimeter tubes. The iodine-containing compounds are dissolved, rinsed out, reduced to inorganic iodine, and measured photoelectrically by their catalytic effect (decolorizing) on a ceric sulfate–arsenious acid solution. This enables quantitative evaluation of the different fractions in the serum (located by standards). Quantities of iodine as low as 0.01γ can be constantly recovered. Recovery of both iodine and thyroxine is in direct proportion to the amount added.

P44. Eosinophil count and glucose level in blood of dairy cattle during the first pregnancy. A. B. SCHULTZE, Univ. of Nebraska, Lincoln.

Eosinophil count and glucose content of blood were determined at 3-wk. intervals in 12 first pregnancy heifers. Although these constituents varied considerably, there appeared to be greater over-all changes during pregnancy than could be accounted for by time or seasonal variation, especially in eosinophil counts.

The mean of eosinophil counts obtained 2 mo. prior to conception and those obtained during the first 2 mo. of pregnancy were used as a basis for evaluating changes during pregnancy. Eosinophil count was 150-200 above base mean value beginning about the 3rd or 4th month of pregnancy and up to about 5½ mo. with a slight decline between 5½ and 6½ mo. From 6½ mo. to shortly before parturition there was a pronounced increase in the number of eosinophils (an av. of 500 more at 8 mo.). At parturition and shortly after there was a sharp drop in eosinophil count (150 less than for the base period), after which the count increased. In general, there was a significant inverse relationship between eosinophil numbers and glucose level during pregnancy and parturition ($r = -0.48$).

These apparent changes may indicate a condition of severe stress at parturition and a state of a minor stress at about 6 mo. of pregnancy.

P45. Interrelationships among plasma 17-hydroxycorticosteroid levels, plasma protein-bound iodine levels and ketosis in dairy cattle. W. G. ROBERTSON, H. D. LENNON, JR., and J. P. MIXNER, New Jersey Agr. Expt. Sta., Sussex.

Seven ketosis cows and 7 control cows, paired as to herd, breed, production level, time since parturition, and time of drawing and processing blood, were used in a study to relate adrenal cortical activity and thyroid activity to the ketotic condition. Ketosis was diagnosed on the basis of clinical symptoms together with low blood sugar and high blood ketone bodies. Plasma 17-hydroxycorticosteroids as an index of adrenal cortical activity were measured by the method of Robertson and Mixner (*J. Animal Sci.*, **13**: 1030. 1954), and plasma PBI as an index of thyroid activity was measured by the method of Brown *et al.* (*J. Clin. Endocrinol. and Metabolism*, **13**: 440. 1953). Plasma 17-hydroxycorticosteroid values averaged 4.81γ% for the normal cows and 7.72γ% for the ketosis cows, and plasma PBI values were 3.58γ and 2.33γ% for the normal and ketosis cows, respectively. In both instances the differences between the mean values were highly significant statistically ($P < 0.01$). High 17-hydroxycorticosteroid values were associated with low PBI values in the ketosis cows. The data suggest that ketosis may be caused by a "relative" adrenal cortical insufficiency induced by low thyroid activity, the elevated blood corticoid values in the ketosis cows representing a failure of utilization or metabolism of the corticoids.

P46. Additional studies on the etiology and treatment of bovine ketosis, including an evaluation of metacortandracin and 9-alpha-hydrocortisone. J. C. SHAW, A. C. CHUNG, and R. A. GESSERT, Univ. of Maryland, College Park.

The new glucocorticoid, 9-alpha-hydrocortisone acetate (Merck), was found to have approximately 15 to 20 times the potency of hydrocortisone alcohol for both the normal and ketotic cow, when evaluated on the basis of its effect on blood glucose, eosinophils, and milk production. One hundred mg. was highly effective for the treatment of bovine ketosis. Metacortandracin (Schering) was found to be 4 to 5 times as effective as hydrocortisone alcohol. Massive doses of hydrocortisone alcohol (5 g.) decreased milk production of normal cows over 60%. Lactate per os was found to be a good supportive treatment and more palatable than propionate. Rumen volatile acids on 3 cows with marked signs of ketosis but relatively good appetites were normal, the total acids ranging from 1,105 to 1,314, μM/ml rumen fluid, and the proportions of acetic, propionic, and butyric acids and higher were 66.8, 18.2, and 15. Observations on 3 additional herds confirm the earlier findings (*J. Dairy Sci.*, **35**: 497. 1952) that the blood eosinophils of normal cows decreased sharply at parturition. Eosinophils of cows with milk fever are usually extremely low or negative.

P47. The effect of synthalin A on blood glucose levels of dairy calves. C. R. RICHARDS and H. G. WEAVER, Univ. of Delaware, Newark.

Secretion of a "hormone" by the alpha cells of the Islets of Langerhans has been reported by a number of investigators. This substance has a hyperglycemic-glycogenolytic effect in the rabbit. The proposal has been made that normal glucose levels in the intact animal are dependent upon a balance of this HGF factor with insulin. A study of these relations in the bovine seems indicated because of the low blood glucose levels in ketosis.

A deficiency of the HGF factor was produced in calves by injecting synthalin A, which has been reported to selectively destroy the alpha cells of the pancreas. After subcutaneous injection of 2.0 mg/kg body wt., the blood glucose rose in 1 hr., dropped about 25% in the next 2 hr. and returned to normal; 2.6 mg/kg resulted in a 10% rise in 1 hr. and a 35% drop in the next 5 hr., which was still evident after 4 days; 4.5 mg/kg resulted in a 40% increase in 3 hr. and a decrease of over 80% in the next 7 hr. (to 10 mg. %), resulting in convulsions 23 hr. post-injection.

P48. The effect of cortisone on the semen of a normal bull. P. T. Cupps, R. C. Laben, and S. W. Mead, Univ. of California, Davis.

Histological studies have indicated that hyperplasia and nodular tumors of the adrenal fasciculata were associated with an increased concentration of spermatozoa and a decrease in fructose concentration. In order to obtain information on the type of adrenal corticosteroids secreted in these cases, a normal bull was treated with cortisone. Semen samples collected during control periods, before and after injections with cortisone, were compared with those collected during the injection period. Cortisone (Cortone, courtesy of Merck & Co.) was injected intramuscularly in 500-mg. doses 3 times weekly for a period of 12 wk. The semen was analyzed for conc. of sperm, motility, % live, % abnormal, fructose and citric acid conc., and total volume. Treatment with cortisone increased the conc. of sperm from 1,046,000 to 1,665,000 per cu. mm. The fructose conc. was decreased from 467 mg. % to 237 mg. %. The increase in conc. of sperm and the decrease in conc. of fructose were significant at the 1% level. None of the other characteristics of the semen were changed during the injection of cortisone.

P49. Response of herd infections of mastitis to various incident factors and therapeutic treatments. L. A. Burkey and Cecelia R. Bouma, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

In early studies *Streptococcus agalactiae* was the predominant infecting organism of mastitis in the dairy herds at Beltsville. Coincident with the increase in machine milking, pseudomonades and hemolytic staphylococci became prevalent after 1938. Later, after infusion treatments with sulfa drugs (1942) and penicillin (1944), the predominant infecting organisms shifted to *S. uberis* and hemolytic staphylococci. The high incidence of infections by coliform bacteria, *S. uberis*, hemolytic staphylococci, and pseudomonades after 1950 were associated with greater dependence on bacteriological diagnosis and routine antibiotic treatments which completely eradicated *S. agalactiae*. Yeast often occurred immediately after penicillin treatments. Currently, cows of a nearby herd with no history of therapeutic treatments for mastitis were heavily infected with *S. agalactiae* but were free of other infecting organisms.

In August, 1953, antibiotic treatments for mastitis were discontinued in the herds at Beltsville except in severe clinical cases. Immediately, all mastitis subsided and mostly disappeared. For 6 mo. largely without antibiotic treatments mastitis cases rarely occurred, and for 18 mo. a relatively low incidence of mastitis occurred. Those found were predominantly streptococcal infections with a marked reduction of infections due to coliform and low-virulent organisms. It is believed that improvements were the results of developed animal resistance.

P50. How hay feeding to cows on pasture affected their milk production, dry matter intake and body weight. D. M. Seath, C. A. Lassiter, Carl Davis, J. W. Rust, and Maurice Cole, Kentucky Agr. Expt. Sta., Lexington.

During the summer of 1954, 8 cows on bluegrass pasture were fed twice daily all of the alfalfa hay they would eat. They were paired in an experiment with 8 comparable cows grazing the same pasture but not fed hay. Both groups were fed a 12% grain mixture according to milk production.

The cows fed hay consumed 11% more dry matter, averaging 29.6 lb. daily as compared to 26.6 lb. for those without hay. Hay feeding apparently reduced by 32% the portion of the dry matter furnished by the pasture—the hay group averaging 11.4 lb. daily from pasture and those not fed hay 16.8 lb.

In spite of the dry summer, both groups of cows maintained a relatively high persistency in milk production. Those fed hay showed no apparent advantage in milk production over that for those not fed hay. There was, however, a slight difference in body wt. gains in favor of the hay-fed group.

P51. The utilization of ammonium nitrate by bromegrass pastures. I. L. Hathaway, Univ. of Nebraska, Lincoln.

Fertilizer studies with ammonium nitrate on bromegrass pastures raised the question regarding the length of time that nitrogen would remain in the soil. Fifty acres of bromegrass were divided into 10 5-acre plots during the spring of 1952 and fertilized with ammonium nitrate at rates which varied from 0 to 740 lb.

of nitrogen per acre. This treatment was repeated again in the spring of 1953. During June, 1954, 1/20 of an acre was cut from each plot, and the dry matter produced per acre was calculated. The data are as follows:

Nitrogen[1] applied	Forage produced	Moisture	Dry matter produced
(lb/A)	*(lb/A)*	*(%)*	*(lb/A)*
0	3,050	73.3	814.1
70	5,700	75.1	1,421.6
140	8,135	74.6	2,063.9
350	21,150	79.5	4,342.1
740	23,325	79.9	4,674.3

[1] The ammonium nitrate was furnished through the courtesy of the Phillips Chemical Co. and the Spencer Chemical Co.

The 10 acres which were unfertilized produced only 4.7 tons of dry matter whereas the 10 acres which were fertilized with 740 lb. of nitrogen per acre during the spring of 1952 and again in 1953 produced 23.3 tons of dry matter. It is evident that much of the fertilizer which had been applied was carried over to the year 1954.

P52. Performance of dairy cows on immature oat forage. G. E. Hawkins, Jr., and K. M. Autrey, Alabama Polytechnic Inst., Auburn.

Milk-stimulating qualities of green oat forage, 6 to 12 in. high, were compared with those of alfalfa hay in 2 trials. Crude protein content of the oat forage ranged from 12 to 27%. Mean digestibility of dry matter (D.M.) of alfalfa hay was 58.8% and that of oat forage was 79.4%, similar to digestibility of the concentrate fed. During the experimental periods, digestible D.M. from immature oat forage was substituted at 0, 50, and 100% levels for digestible D.M. allowances provided by alfalfa hay during the preceding standardization period. Persistency index of milk flow in Trials 1 and 2 was 88%, 99%, and 99%, respectively, for the 0, 50, and 100% levels of oat forage. Production of FCM per lb. of digestible D.M., above maintenance requirements, was similar for all diets. The greater milk flow of cows on 50 and 100% levels of oat forage than on alfalfa hay was related to greater digestible D.M. intake. Increased intakes of digestible D.M. probably account for the increases in milk production when half of the cows in Trial 2 were turned to pasture.

P53. Influence of grazing on persistency of ladino clover. J. W. Cobble, I. H. Stuckey, and B. W. Henderson, Jr., Rhode Island Agr. Expt. Sta., Kingston.

Maintenance of ladino clover in pastures is a major problem. The influence of grazing on ladino clover was studied with 15 half-acre plots, 14 grazed plus 1 control. Comparable groups of dairy heifers were used. Four systems of management were followed: intensive grazing, less intensive grazing, silage followed by intensive grazing, and silage cut followed by less intensive grazing. Original seeding of the pasture was made in 1952 with 2 lb. ladino clover, 3 lb. orchard grass, and 1½ bu. oats. Data included wt. gains of the heifers, total acreage yield, botanical composition, heifer-days pasturing. Body wt. gains were not significantly affected by management. Total yield for intensive grazing in 1953 was 5.50 tons of hay equivalent containing 51.8% clover. In 1954, total yield was 4.88 tons with 17.8% clover. First year yields for less intensive grazing were 6.46 tons total hay equivalent with 53.1% clover; 2nd year yields averaged 5.48 tons containing 11.3% clover. Mean clover yields expressed as tons of hay equivalent for both years according to the 4 management practices investigated were: intensive grazing, 0.72; silage followed by intensive grazing, 0.99; less intensive grazing, 0.54; silage followed by less intensive grazing, 0.68.

P54. Comparison of alfalfa hay and wilted alfalfa silage as a roughage for growing dairy heifers. J. F. Sykes, L. A. Moore, and H. T. Converse, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Alfalfa hay was compared with wilted alfalfa silage or with alfalfa silage and limited alfalfa hay as roughages for dairy heifers reared to 2 yr. of age in a feeding system in which milk was discontinued at 60 days of age and concentrate feeding was discontinued after 8 mo. of age, using a total of only 400 to 500 lb. of concentrates. The wilted alfalfa silage was made without preservatives. Satisfactory rates of growth were obtained when alfalfa hay provided the only roughage. Under similar conditions heifers made subnormal gains when wilted alfalfa silage was the only forage fed. When wilted alfalfa silage and a limited amount of hay were fed together, growth rates were improved as compared to alfalfa silage alone but were still unsatisfactory. The growth rates obtained on these forages were largely a reflection of feed intake. It is suggested that the reduced intake on the alfalfa silage rations was due not so much to insufficient capacity as to a lack of appetite for silage. The data indicate that in order to utilize alfalfa silage for growing heifers in a limited grain feeding system a higher proportion of nutrients must be provided from other forages or concentrates than have been used in this experiment, particularly during the 1st yr. of age.

P55. A comparison of results obtained by different measures used in studying temporary summer pastures. L. M. Underwood, W. J. Miller, and T. H. Rogers, Univ. of Georgia, Athens.

Comparable groups of lactating dairy cows rotationally grazed 3 areas of Tift Sudan and

3 of Starr millet. Each complete rotation of 3 wk. served as a period in a double reversal design in which the cows were switched to the opposite species at the end of each period. The Sudan exceeded the millet in the following measures: FCM, 2.2%; persistency, 7%; wt. gain, 0.5 lb/cow/day; TDN yield as determined by animal requirements, 5%; TDN yields as determined by inside-outside cage differences, 15%; and TDN as determined by a cage method correcting for differential growth rate, 15%. The millet exceeded the Sudan by 9% in actual cow-days grazed. There was no difference in the fat percentages of the milk but the percentage apparently was reduced somewhat below normal by both species. The av. yields of TDN per acre were 1,326 lb. by the animal requirement method, 1,544 lb. by the inside-outside differences method, and 1,280 lb. by the cage method correcting for differential growth rate. None of the differences observed were statistically significant ($P < 0.05$).

P56. Effects of various hay:concentrate ratios on production responses and nutrient utilization of dairy cows. S. Bloom, N. L. Jacobson, L. D. McGilliard, E. O. Heady, and P. G. Homeyer, Iowa State College, Ames.

Hay and concentrates in 4 ratios (75:25, 55:45, 35:65, 15:85) were fed to lactating cows for 182 days, following a 60-day preliminary period, in isodynamic amounts above individual maintenance requirements at each of 3 feeding levels (high, medium, low). Thirty-six Holsteins were divided into high-, medium-, and low-producing groups, and 3 cows, 1 of each group, were placed at each of the 12 "hay:concentrate-feeding level" positions. For each cow the total feed energy requirement for production was predetermined and partitioned into decreasing weekly quantities. Feeding was based on net-energy recommendations. Ration digestibility was determined for each cow by the chromic oxide technique.

Production of 1 lb. of FCM from high-hay to low-hay rations, respectively, required 0.44, 0.40, 0.38, 0.33 lb. TDN as compared to 0.36, 0.36, 0.37, 0.35 therms ENE. Weight changes during the initial 5 wk. were closely related to diet but thereafter were largely independent of ration. Differences in ENE available for milk production at the 3 feeding levels were much greater than the corresponding differences in FCM production. Dry matter digestibilities for high-, medium-, and low-ability animals were 58.2, 58.2, and 57.2% and for high-, medium-, and low-feeding levels were 57.6, 57.8, and 58.2%.

P57. Comparative grazing performance of purebred Brown Swiss, Brown Swiss × Red Sindhi crossbreds, and Jersey cows under Louisiana conditions. E. J. Stone and D. M. Johns,[1] Louisiana Agr. Expt. Sta., Baton Rouge.

Twenty-two Brown Swiss, 7 Brown Swiss × Red Sindhi, and 13 Jersey females were observed for 21 consecutive days, 24 hr. per day, to relate their grazing performance with climatic conditions and milk production. Mean values for climatic conditions during the period studied were: 92° F. and 26 mm. Hg. vapor pressure. The crossbreds grazed more in the sun than the purebred Swiss and Jerseys, whereas at night the Jerseys grazed more than the Swiss and the crossbreds, in that order. Total daily grazing time was greatest for the crossbreds and least for the Jerseys. Mean body temperatures for the period were: crossbreds, 101.7° F.; Brown Swiss, 102.1° F., and Jerseys, 102.2° F. Mean respiration rates by breeds were: crossbreds, 49; Brown Swiss, 73; and Jerseys, 66. Av. daily milk production in lb. FCM was: Brown Swiss, 15.9; Jerseys, 15.0; and crossbreds, 14.1.

[1] North Louisiana Hill Farm, Homer, La.

P58. A comparison of an immature corn silage with alfalfa hay as the forage for dairy cows. E. A. Keyes and E. P. Smith, Montana Agr. Expt. Sta., Bozeman.

Twelve dairy cows, 6 Holsteins, and 6 Jerseys, were divided into 3 equal groups. Grp. I was fed immature corn silage as the sole roughage; Grp. II, corn silage and alfalfa hay in combination; and Grp. III, only alfalfa hay as a roughage. All groups were fed the same grain mixture according to production. Grp. I gained the most weight—283 lb. compared to 81 and 70 lb. for Grps. II and III, respectively.

Grp. II (silage) produced 10,860.9 lb. of FCM, Grp. III (alfalfa hay) 10,542 lb., and Grp. I (corn silage) 9,512.5 lb. during the experiment. Considering efficiency of production, Grp. I ranked first by producing 1.69 lb. of FCM per lb. of TDN consumed, compared to 1.58 and 1.31 lb. of FCM per lb. of TDN for Grps. II and III, respectively.

Considering alfalfa hay at $20 and corn silage at $4.50 per ton, the forage cost of producing 100 lb. of FCM would be $0.72 for silage, $0.84 for silage and alfalfa hay in combination, and $1.08 for alfalfa hay.

P59. Self-feeding of Bermuda grass hay-grain mixtures to lactating cows. F. N. Baker, North Louisiana Hill Farm Expt. Sta., Homer.

Self-feeding of 3 hay-grain mixtures was compared with hand feeding of grain. Experiments involved 8 Jersey cows in late lactation in two 4 × 4 Latin squares. Comparisons were based on 10-day periods following 4-day change-over periods. All cows received all the oat silage they would eat in addition to grain hand-fed or hay-grain mixtures self-fed. The self-fed rations were composed of coarsely ground Coastal Bermuda grass hay and a stock

15% dairy ration, the latter comprising 25, 35, and 45% of the mixtures.

There were no significant differences in production of FCM or body wt. changes attributable to rations, indicating that all of the mixtures tested were physiologically suitable for feeding. Highly significant differences between rations were found in consumption of grain and silage. Total grain consumption increased with increasing percentages of grain in the mixture. Silage consumption declined with increases in consumption of the hay-grain mixtures. The self-fed mixtures all provided protein and TDN in excess of the requirements for maintenance and production according to the Morrison standards.

P60. The effect of nursing calves on milk production of identical twin heifers. E. W. SWANSON, Univ. of Tennessee, Knoxville.

Six comparisons have been made in which identical twin heifers were allowed to nurse their calves until vealing size while their mates were milked and their calves hand-fed. Three of the heifers were stripped after nursing. At vealing age, averaging 65 days, the milked twins had produced an av. of 1,536 lb. vs. 146 lb. obtained from their mates. In the 1st wk. after vealing the milked and nursed cows averaged, respectively, 22.9 and 17.8 lb. of milk daily. At 15 wk. of lactation they averaged, respectively, 20.3 and 18.6 lb. of milk daily, and their total production averaged 2,460 and 963 lb. The twin pairs were nearly equal in production at an av. of 6 to 7 wk. after vealing. Individual pairs required from 2 to more than 10 wk. to attain equal production. Thus, the full cost of the suckled veal was about 1,500 lb. of milk compared to 1,179 lb. given to the fed calves. The suckled veals gained 1.95 lb. daily compared to 1.82 lb. for the fed calves. The suckled veal graded high good compared to low good for the fed calves, and was sold for 1.3¢ more per lb.

P61. A sanitary study of detergents and detergent-sanitizers used in the circulation cleaning-in-place of a pipeline milker installation in a stanchion dairy barn. J. B. LINDAMOOD, E. J. FINNEGAN, and G. C. GRAF, Virginia Polytechnic Inst., Blacksburg.

An investigation was conducted to ascertain the sanitary aspects of several detergents and detergent-sanitizers used in cleaning 388 ft. of 1½ in. Pyrex glass pipeline in a stanchion dairy barn. The circulation vacuum cleaning method was used.

The detergents and detergent-sanitizers were used in conjunction with acid cleaners and sanitizing agents as directed by the respective manufacturers, with the exception of the temperature ranges cited below. The cleaning program was divided into a "cold" circulation period with a temperature range between 140° and 105° F. and a "hot" period between 160° and 130° F. The hot range was controlled by a 5,000-w. heater in an insulated circulation tank. The products and directions of 6 detergent manufacturers were studied each for one 15-day hot period and one 15-day cold period. Swab samples were taken at appropriate locations 3 times during each period.

Preliminary results of a survey covering 6 mo. indicate that there is more variation between the cleaning ability of detergents than between a higher and lower circulation temperature with the same detergent.

P62. Normal variations in rate of milking.[1] W. E. STEWART and L. H. SCHULTZ, Cornell Univ., Ithaca, N. Y.

The purpose of this study was to determine the amount of variation in milking rate which could be expected when a proper milking procedure was followed. The study covered 4 yr. and involved 286 cows of 4 breeds in the Cornell herd. Rate of milking measurements were made by suspending the milker pail from a scale and taking readings every 15 sec. during the milking process. Machine stripping was started when the rate of flow declined to a level of 0.3 lb. or less between readings. The machine was removed when milk flow stopped.

For the 286 cows averaging 2.75 lactations and 154 days in milk, the av. total milking time was 3 min. and 33 sec. The av. amount of milk obtained was 13.5 lb., which resulted in an av. rate of flow of 3.85 lb. per min. Machine stripping took 37 sec. and resulted in an av. of 1.5 lb. of milk.

There were differences in rate of milking due to stage of lactation. For 98 cows in 3 successive stages of lactation, the av. total required milking time was 4 min. 28 sec. in early lactation, 3 min. 25 sec. in middle lactation and 2 min. 49 sec. in late lactation.

Total milking time for individual cows varied from 1 min. 15 sec. to 11 min. 40 sec. Two hundred thirty-one cows milked in midlactation (153 days av.) required the following milking times: under 3 min., 35%; 3 to 4 min., 38%; 4 to 5 min., 18%; over 5 min., 9%.

[1] Supported in part by the DeLaval Separator Co., Poughkeepsie, N. Y.

P63. Preliminary report comparing cellular antigens with type defects in dairy cattle. P. G. NAIR, T. M. LUDWICK, E. J. LAZEAR, and L. C. FERGUSON, The Ohio State Univ., Columbus.

Daughters of several bulls were classified independently by 2 different classifiers at 3 mo. after calving. Animals were evaluated on a complete breakdown, and each division of the score card was further divided into numerous type defects. Defects which occurred rarely were omitted from the intrasire and intraherd studies. The animals were blood typed at Ohio

State Univ., and the types were grouped according to the inheritance pattern.

Variations for type defects were studied for each group of antigens, and the Chi square test was adopted to test for homogeneity between animals having particular antigens and those that did not. In these animals no particular defect was correlated with any antigen. In each family, however, the presence or absence of certain antigen groups was associated with the frequency of occurrence of type defects. When data from all families were combined for analysis, different antigen groups were again associated with the frequency of certain defects. The authors do not suggest any generalization on the basis of these results.

P64. Estimated changes in the environment and average real producing ability in a Holstein herd from 1901 through 1954. W. M. DILLON, JR., W. W. YAPP, and R. W. TOUCHBERRY, Univ. of Illinois, Urbana.

Maximum likelihood estimates of environmental changes and average real producing ability by years were based on the method developed by C. R. Henderson. The records used were from the Univ. of Illinois dairy herd, which contained 578 cows with 1,656 records; these were corrected to a 305-day, 2X, ME, FCM basis. The regression of environmental change on years was 53.54 ± 14.44 lb. of FCM. The av. change in environmental effect between years was 534 lb. with extremes of +2,413 and -1,703 lb. of FCM. The regression of av. real producing ability on years was 0.68 ± 14.00 lb. of FCM. The mean change in av. real producing ability between years was 706 lb. with extremes of +2,067 and -1,865 lb. of FCM.

There was an av. of 17 records/yr over the first 27 yr. and 45 records/yr over the last half of the study. An av. of 7 cows was included in each birth yr. group through 1927. From 1928, these groups included an av. of 14 cows. These results indicate that environmental changes from year to year can greatly influence a cow's production. Furthermore, the average real producing ability in this herd has changed very little since it was founded.

P65. The relationships between heritability and twin efficiency values calculated from twin uniformity trials. R. LAIRD and L. O. GILMORE, Ohio Agr. Expt. Sta., Wooster.

A formula for heritability, H (Thoele and Hervey, 1952), and a formula for twin efficiency value, E (Carter 1951), are based on the mean squares between twin-pairs and within twin-pairs in the analysis of variance of twin uniformity trials. Since both these formulas have the same basis, it is apparent that they must be related. However, the literature on the subject does not reveal any formula directly relating the 2 terms. From the original formulas the following relationships were derived:

$$H = \frac{E - 1}{1}$$

$$E = \frac{1}{1 - H}$$

As heritability increases, the efficiency of monozygotic twins for experimental purposes is demonstrated to increase at an accelerating rate until a value of infinity is obtained with heritability estimates of 100%.

An analysis of growth data on 6 pairs of monozygotic dairy and dual-purpose heifers at 9, 12, and 15 mo. of age was undertaken to estimate heritability and twin efficiency. Only 9 of 54 heritability estimates were lower than 90%. Head length, head width, body length, thurl width, hooks width, and body wt. had mean heritabilities over 95%, whereas pins width, pelvis length, shoulder width and heart girth fell into the range 90-94%, and withers ht. and chest depth were 87%.

P66. Physical changes in young dairy heifers as indicated by type evaluation studies. C. M. CLIFTON and F. ELY, The Ohio State Univ., Columbus.

The type of 544 Holstein heifers in 5 herds was scored at 3, 6, and 12 mo. of age. The score card was divided into 10 parts with equal point value as follows: head and neck, shoulders and chest, middle and loin, rump and thighs, feet and legs, size and shape of udder, udder attachments, teats, dairy character, and general appearance. The scores used were, in most cases, the av. rating of 2 judges. The repeatability of ratings from one age to another in the same heifer was estimated by linear correlation. These estimates ranged from 0.16 to 0.37 for different score card divisions and age comparisons.

Age differences were significant at the 1% level in all score card divisions. The most notable and consistent of these differences were: (1) progressive improvement in shoulders and chest, (2) improvement in feet and legs, and (3) a downward trend in scores for rump and thighs. Sire differences were detected in each herd in which 2 or more sires had 20 daughters. Each score card division and each age were represented in these differences. The most frequent and persistent differences were in rump and thighs, feet and legs, and udder.

P67. A type and production study of Holstein-Friesian cattle in Canada. J. B. STONE, J. C. RENNIE, and G. E. RAITHBY, Ontario Agr. College, Guelph.

A sample of 1,500 cows (300 from each of the 5 type classes Excellent to Fair) and all of their daughters (1,037) were used to estimate the phenotypic correlation between type and production, the repeatability of production records, and the heritability of type ratings and production records. Production was expressed

on a 6 yr., 2X, 305-day basis for milk, fat, and test, and in addition milk and fat yields were expressed as percentages of the breed class average. The analyses were made on an intrasire basis.

The phenotypic correlations between type and producing ability, as calculated by the av. of all records of a cow were 0.25 for mature equiv. milk, 0.19 for mature equiv. fat, 0.22 for breed class av. for milk, 0.21 for breed class av. for fat, and 0.02 for test.

The repeatabilities of the various measures of production were mature equiv. milk, 0.50; mature equiv. fat, 0.52; breed class av. for milk, 0.51; breed class av. for fat, 0.52; and test, 0.59.

The following heritabilities, calculated from the regressions of daughter on dam, were obtained: type rating, 0.21; mature equiv. milk, 0.25; mature equiv. fat, 0.27; breed class av. for milk, 0.24; breed class av. for fat, 0.25; and test, 0.35.

P68. An analysis of the components of type of Holstein-Friesian cows in Canada. J. C. RENNIE and G. E. RAITHBY, Ontario Agr. College, Guelph.

All cows officially classified for type during 1954 by the Holstein-Friesian Assoc. of Canada were used to determine the distribution of type scores for final rating and for each of 4 main sections of the type score card and of 4 subsections. Phenotypic correlations between final rating and each type component were based on an intraherd and intrasire analysis of single type scores. The 12,889 cows were distributed in 1,730 herds and by 3,628 different sires. Of these cows, 53.5% were 1st calf heifers and 94.5% were 1st inspections.

The percentage of the cows classified "Good Plus and Better" on final rating and on each type component was as follows: final rating, 48.8; general appearance, 52.8; dairy character, 81.3; body capacity, 79.2; mammary system, 50.4; fore udder, 48.8; rear udder, 57.0; legs and feet, 45.2; and rump 45.7.

The phenotypic correlations between final rating and each type component were as follows: general appearance, 0.822; dairy character, 0.499; body capacity, 0.334; mammary system, 0.794; fore udder, 0.665; rear udder, 0.648; legs and feet, 0.438; and rump 0.503.

P69. An evaluation of the American Jersey Cattle Club star bull program. W. J. BRAKEL, The Ohio State Univ., Columbus.

The star bull program was designed to serve as a guide in the evaluation of the pedigrees of unproved Jersey sires. The number of stars awarded a sire is intended to serve as an index of his genetic worth. An attempt to evaluate the merits of this program was made by determining the mean butterfat production and type classification rating of the progeny of the 1 to 7 star bulls. The progeny performance of the sires in the various groups was determined by comparing the 1,141 bulls contained in the 1944-1949 Tested Sire Reports with the 15,109 star bull certificates issued during the 6 yr. subsequent to Jan. 1, 1943.

The regression of the daughters' mean butterfat production on their sires' star rating was 8.62 lb. with 5% fiducial limits of 4.30 to 12.94 lb. This regression was linear and significant at the 1% level. No real difference was found between the av. type classification scores on the progeny of the sires in the 1 to 6 star groups.

P70. Udder palpation in heifers as a basis for estimating their milk yield as cows. W. W. YAPP, W. M. DILLON, JR., and W. R. SMITH, Univ. of Illinois, Urbana.

In 1946 the Dept. of Dairy Science began a study of dairy heifer development that included udder palpation. In this project each heifer calf born in the University dairy herd was first palpated when 2 wk. of age and at each 2-wk. interval thereafter, until it was deemed that the separate quarters had fused into a smooth mass. Of the heifers palpated, 211 have calved and concluded one or more lactations. Five breeds of dairy cattle have been included in the study.

It has been found that the coefficient of correlation between the fat-corrected milk (FCM = 0.4M + 15F) and the palpation score at 4 mo. of age for each breed is as follows: Ayrshire, $r = 0.32$; Brown Swiss, $r = 0.56$;[1] Guernsey, $r = 0.08$; Holstein-Friesian, $r = 0.05$; and Jersey, $r = 0.28$. On the same basis the within-breed correlation is $r = 0.14$ at 4 mo., $r = 0.11$ at 3 mo., and $r = 0.01$ at 6 mo. The within-breed correlation between ht. at withers and FCM is 0.21[1] at 3 mo. and 0.10 at 6 mo. of age.

A multiple correlation based upon all palpation scores at the different ages gives a value of $R^2 = 0.05$; similarly, all ht. measurements give a value of $R^2 = 0.05$.

[1] Significant at the 1% level.

P71. Phenotypic relationships among climatic conditions and performance of lactating cows. J. E. JOHNSTON, G. D. MILLER, J. B. FRYE, JR., and J. J. VIZINAT, Louisiana Agr. Expt. Sta., Baton Rouge.

Thirty-eight Holstein, 23 Jersey, and 8 Red Sindhi-Holstein (F_1) cows were studied for 12 hot days (mean DBT 91° F.). Three P.M. body temperatures and respiration rates and production of FCM were recorded daily. Day and night grazing times were recorded on 8 and 5 days, respectively. Air temperature and humidity (vapor pressure) were recorded at time of observation, as well as 9:00 A.M.-3:00 P.M., and 9:00 P.M.-3:00 A.M. (previous night) mean temperatures. Within-breed correlations between climatic conditions and animal reactions indicate that under the conditions of this study

previous night temperature was of greatest importance in determining body temperature and respiration rate, and vapor pressure was most closely related to milk production. Correlations tended to be highest in Holsteins and lowest in crossbreds. Total among- and within-cow correlations were calculated within breed among the animal measurements. Correlations between body temperature or respiration rate and milk production or grazing performance were highest in Holsteins and lowest in crossbreds. Among-cow correlation between body temperature and milk production was +0.50; however, within-cow correlations were negative. Among- and within-cow regressions of milk production on body temperature in Holsteins were 6.2 and −1.5, respectively.

P72. Effect of heavy concentrate feeding prior to calving upon lactation and upon mammary edema. K. E. GARDNER and J. F. D. GREENHALGH, Univ. of Illinois, Urbana.

Beginning 6 wk. prior to calving, 9 cows were fed increasing amounts of concentrates at a daily rate of 5, 7, 9, 10, 11, and 12 lb. through the 1st wk. prepartum, respectively. Nine heifers received 4, 5, 6, 7, 8, and 9 lb. of concentrates for the same weeks. Alfalfa hay was fed at 1.5 lb. per 100 lb. body wt. and corn silage at 2 lb. The control group composed of 8 cows and 9 heifers received roughages at the rates stated but were fed no concentrates prior to calving. For 84 days after calving both groups were fed concentrates sufficient along with roughages to meet Morrison's optimum TDN standard.

No significant difference was observed in milk production between the 2 groups during the first 84 days after parturition. The incidence and severity of mammary edema as gauged by both visual ratings and linear measurements were not significantly affected by the heavy grain feeding. The first calf heifers showed significantly more edema than did the cows ($P < 0.01$). Body wt. was significantly increased during the dry period as a result of heavy grain feeding ($P < 0.05$).

P73. The resistance of milk samples from cows on silage to the action of S. agalactiae. W. D. POUNDEN, NORMA A. FRANK, R. W. BROWN, and R. K. SCHERER, Ohio Agr. Expt. Sta., Wooster.

Measurement of the resistance of milk samples to the action of *S. agalactiae* may offer a means for estimating the relative resistance of cows to the infection. Variations between samples from cows on different rations have been encountered, and the present report is a continuation of these studies.

A marked loss in resistance of milk samples followed on addition of corn silage to a ration of mixed hay and grain being fed a group of 7 cows, none of which had eaten any silage or pasture for at least 18 mo. The loss in resistance persisted for 1 mo. and was followed by a level of resistance that was unusually high for the group. Similar marked losses in resistance were not encountered in cows that had been on pasture during previous months.

A ration of alfalfa hay, forage-crop silage, and moderate grain feeding was associated with maintenance of a satisfactory level of resistance in 10 cows continuously observed, using weekly collected milk samples, for most of their lactation periods.

P74. A comparison of milk yields and estimated secretion rates when between-milking intervals were varied. J. D. DONKER and H. L. DALTON, Univ. of Georgia, Athens.

Milking schedules used: 3 A.M.-3 P.M., 4 A.M.-3 P.M., and 5 A.M.-3 P.M. To estimate milk secretion rates as differentiated from observed yields, complementary milk was removed by using 15 IU oxytocin intravenously. Six cows had their complementary milk measured 2 mornings and afternoons, spaced to minimize lactation trends and injection effects. Six other animals were treated at 4 consecutive milkings. The total milk secreted in an interval was arrived at by adding the complementary and the normal milk obtained at the end of the interval and eliminating the complementary milk present at the start of the interval. In the spaced-injection series, the complementary milk present at the start of the interval was estimated from independent observations and had to be subtracted from the total. In the consecutive-injection series, the complementary milk was actually removed prior to starting the interval.

The afternoon milk yields for each 2 wk. prior to injections were, on a time unit basis, 6.0 to 8.0% above the morning milkings and were independent of milking schedule. The secretion rates for both injection series showed less than 2.0% difference between the night and day rates and also were independent of the length of interval.

P75. "Subnormal milk"—its production and correction. R. B. BECKER, P. T. DIX ARNOLD, J. M. WING, JACK MCCALL, and G. K. DAVIS, Florida Agr. Expt. Sta., Gainesville.

Low-fiber concentrates and a bulky concentrate mixture of dried brewers' grains and citrus and beet pulps were fed to meet the Morrison protein and TDN requirements of 10 Jersey and Guernsey cows in medium to good milk flow. Creamline color diminished within 10 to 14 days. Fat percentage dropped gradually 0.5 to 2.2% below that of the basal period, but SNF dropped only slightly. The cows' craving to chew pine wood was barred by an electric fence. Consumption of mineral supplement offered ad lib. increased.

In the recovery period, 2 cows receiving 15 lb. of corn silage daily and 2 cows on pasture gradually returned to nearly normal fat test. A slower response occurred with 2 cows receiv-

ing 4 lb. of Pangola grass hay daily, and no significant response resulted from feeding 2 cows 1 lb. of alpha cellulose daily. Responses appeared to be independent of carotene and of alpha cellulose in the feed. Samples of feeds and rumen liquid are being analyzed. Other changes concerning character of milk are cited in paper M21.

Under field conditions, "subnormal milk" production was observed from cows on succulent white clover and on young winter oats pastures.

P76. Bromide content of milk when cows are fed forage grown on ethylene dibromide-treated soil.[1] R. W. YOUNG, L. I. MILLER, W. A. HARDISON, and R. W. ENGEL, Virginia Agr. Expt. Sta., Blacksburg.

Fumigation with ethylene dibromide is effective in controlling sting nematode *(Belonolaimus gracilis Steiner)* soil infestation in the peanut-growing areas of Virginia. Peanut hay grown on check plots and on plots treated with 3 effective levels of ethylene dibromide was fed to dairy cows at various stages of lactation to determine effects on milk bromide level.

The 25 to 60 p.p.m. of bromide found in peanut hay from untreated plots increased milk bromide levels to about 20 p.p.m., or approximately 3 times that found in the milk of cows fed a standard dairy ration, roughages, supplement, and silage grown at Blacksburg. Feeding peanut hay from fumigated plots (bromide content of 243 to 352 p.p.m.) produced a steady rise in milk bromide content. Levels approaching 100 p.p.m. resulted after 4 wk. of feeding.

Feeding peanut hay from fumigated plots in a standard dairy ration (about 15 lb. of hay, 30 lb. of silage, and 1 lb. of supplement per 4 lb. of milk produced) resulted in milk bromide levels only slightly higher than those observed when peanut hay from check plots served as the only ration ingredient.

[1] This work was supported in part by a grant from the Dow Chemical Co., Midland, Mich.

P77. The secretion of I^{131} in milk. F. W. LENGEMANN, R. A. MONROE, and E. W. SWANSON, UT-AEC Agr. Research Program, Oak Ridge, Tenn.

A study has showed that about 5% of a dose (either oral or I.V.) of I^{131} was found in the milk within 7 days. The extremes were 1.43 to 16.4%. The total % of I^{131} in milk seemed to be independent of the level of production. Dietary iodide caused only a small variation in the I^{131} content of milk. Increasing the iodide intake from 20 mg. to 2,000 mg. per day decreased the I^{131} content of milk of one cow from 4.6 to 3.0%. About 0.02% of an oral dose was found in milk 30 min. after administration, indicating that iodide is rapidly absorbed through the rumen wall.

The kidneys appeared to be the main pathway of excretion with about 2/3 of the I^{131} being found in the urine. Results from an I.V. dose duplicated those of the oral dose. This may indicate that nearly all of an oral dose of iodide is absorbed into the body and then part returned to the gut.

P78. The acid-soluble nucleotide content of mammary gland and of tissues from animals fed galactose. R. G. HANSEN and R. A. FREEDLAND, Univ. of Illinois, Urbana.

The importance of uridine diphosphate hexose in galactose and lactose metabolism prompted a study of tissues nucleotides. Levels of the various nucleotides have been estimated in perchloric acid extracts of guinea pig liver, guinea pig mammary gland, liver from chicks fed a normal diet, and liver from chicks fed 15% galactose. Uridine diphosphate hexose and other acid-soluble nucleotides have been separated in the tissue extracts by ion exchange chromatography. Fifteen to 20μM of uridine diphosphate hexose per 100 g. have been isolated from the normal tissues. These studies indicate that the level of uridine diphosphate hexose is similar in a variety of normal tissues.

Feeding galactose to chicks increased the level of uridine diphosphate hexose in the liver. This confirms previous estimates made by enzymic assay. The uridine diphosphate hexose was hydrolyzed and the carbohydrate component identified by paper chromatography. In the normal tissues, uridine diphosphate hexose contained galactose and glucose in an approximate ratio of 25 to 75, whereas in the tissues from chicks fed galactose this ratio was reversed. Possible importance of these observations in the metabolism of galactose and lactose by mammary gland tissue will be discussed.

P79. Milk protein production in the bovine. B. L. LARSON, G. D. ROLLERI, and K. A. KENDALL, Univ. of Illinois, Urbana.

Production level of the milk proteins was evaluated during the entire lactation period of several cows by standard methods and the specific proteins by a quantitative electrophoretic procedure. In contrast to the normal increase during approximately the 1st mo. in the lb. of milk and fat produced per day, the total amount of protein produced per day tended to remain constant or to decrease. Thus, the cows were in full protein production at the time of parturition. During the first few milkings after parturition, however, the fractions comprising the total proteins changed with an increase in α- and β-casein offsetting the decrease in the immune globulins. After this decrease the production per day of the immune globulins tended to remain fairly constant over the lactation period as also did serum albumin and γ-casein. In contrast to these proteins, α- and β-casein, β-lactoglobulin, and α-lactalbumin decreased steadily.

Comparative studies on the mammary fluid of dry cows and the milk of cows milked without

a normal drying off period, indicate that some specific proteins are always present in the mammary gland, whereas others are notably present only when the cow is being milked or undergoing the stimulus of nearing parturition time.

P80. The fat and solids-not-fat content of milk of individual cows. T. N. COMBS, P. M. REAVES, and G. C. GRAF, Virginia Polytechnic Inst., Blacksburg.

One-day composite samples of milk from individual cows were obtained at monthly intervals over an 11-mo. period. These included 505 samples from Holsteins, 279 from Guernseys, and 234 from Jerseys. The samples were analyzed for % fat and TS, with the % SNF being calculated by difference. The av. composition of the milk of the three breeds in this study is: Holstein, 3.78% fat and 8.46% SNF; Guernsey, 5.28% fat and 9.18% SNF; Jersey, 5.86% fat and 9.49% SNF.

Data are included on the effect of season and the relationship of the % of fat to the % SNF.

P81. A preliminary report on the optimum protein level of calf starters. L. D. BROWN, C. A. LASSITER, and J. W. RUST, Univ. of Kentucky, Lexington.

Forty 2-day-old male and female calves were used in studies to determine the optimum protein level of calf starters. All calves were raised to 84 days of age on a limited milk-hay-starter system.

Four calf starters were prepared with varying levels of crude protein. Starter I averaged 24.3%, Starter II, 20.2%; Starter III, 16.6%; and Starter IV, 12.2% crude protein. An alfalfa-bromegrass hay was fed which contained 13.4% crude protein. The calves were divided into 4 equal groups (5 Jerseys and 5 Holsteins each) and fed one of the 4 starters ad libitum with an upper limit of 4 lb. daily for Jersey and 5 lb. for Holstein calves. Digestion nitrogen balance, and blood studies were conducted in addition to the collection of growth data.

There were no significant differences between the 4 protein levels as to growth; however, significant differences were obtained in efficiency of feed utilization. The av. daily gains (lb.) were 0.84, 0.92, 1.00, and 0.98 for Starters I, II, III, and IV, respectively. In the same order the efficiency of feed utilization values (lb. feed/lb gain) were 3.12, 3.21, 2.91, and 2.89. The av. starter consumption was 138.1, 159.3, 159.2, and 160.1 lb. for Starters I, II, III, and IV, respectively. The av. hay consumption for the various groups was similar. The blood serum protein levels of the calves were not significantly affected by the level of protein in the starters.

P82. Interrelationships between carotene from artificially dehydrated alfalfa and vitamin A from a dry carrier when fed simultaneously to Holstein calves. K. L. DOLGE, J. E. ROUSSEAU, JR., R. TEICHMAN, H. D. EATON, and G. BEALL, Conn. Agr. Expt. Sta., Storrs.

Forty-eight 63-day-old male Holstein calves, which had been partially depleted of their vitamin A stores, were fed a vitamin A depletion ration containing one of 4 levels of vitamin A from a dry carrier: 4, 10, 25, or 62.5γ/lb of live wt. per day and one of 4 levels of carotene from artificially dehydrated alfalfa leaf meal: 20, 50, 125, or 312.5γ/lb of live wt. per day for a 12-wk. period. At the end of this period the animals received the unsupplemented depletion ration for 4 wk. so that body stores of vitamin A might be estimated. All animals were then slaughtered. Analysis of the data for the last 8 wk. of the supplement feeding period indicated the following: *(a)* Plasma and liver vitamin A were linearly related to the log of both the carotene and vitamin A intake, *(b)* plasma carotene was significantly suppressed by high levels of vitamin A intake and liver carotene only slightly so, *(c)* plasma carotene responded linearly to the log of carotene intake, and *(d)* body stores of vitamin A, as estimated by depletion time, were linearly related to the log of both carotene and vitamin A intake.

P83. Plasma tocopherol levels of dairy animals receiving different diets. J. W. THOMAS and M. OKAMOTO, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Blood plasma tocopherol determinations on dairy calves and cows have been made by using a modified hydrogenation procedure developed by Quaife and Biehler. The plasma tocopherol level was generally related to the tocopherol intake. The level tended to increase with age of the calves. Both cows and calves receiving silage or green forage had higher plasma tocopherol levels than similar animals receiving alfalfa hay. Calves receiving only whole milk and calves receiving skimmilk, grain, and alfalfa hay had values of approximately 0.12 mg. tocopherol per 100 ml. plasma. The addition of grain or alfalfa to the whole milk diet about doubled the plasma level. Calves fed a synthetic milk with lard as the fat source had comparably low plasma tocopherol levels. When tocopherol was added to the synthetic milk to supply 10 or 20 mg. tocopherol per cwt. the plasma values averaged about 0.05 and 0.08 mg. %, respectively. Calves fed these supplemented levels for a period of 3 to 6 mo. developed symptoms of vitamin E deficiency. The administration of 50 mg. tocopherol per day by capsule to calves fed synthetic milk or whole milk resulted in plasma tocopherol levels of 0.30 and 0.50 mg. %, respectively, and prevented vitamin E deficiency symptoms. Diarrhea in calves caused a marked decrease in plasma tocopherol level and prevented increases in plasma levels when tocopherol was given by capsule.

P84. Study of a pregastric esterase observed in the alimentary tract of the calf. H. A. RAMSEY, G. H. WISE, and S. B. TOVE, North Carolina State College, Raleigh.

An investigation of pregastric esterase in the calf was initiated with the following objectives: to identify the tissues that secrete pregastric esterase, to study the effect of age of animal on the esterolytic activity of these tissues, and to ascertain the substrate specificity of this enzyme system.

Extracts (filtrates of tissue homogenates) of *(a)* the tongue, region of the vallate papillae, *(b)* the lining of the glosso-epiglottic space, *(c)* the esophagus, pharyngeal end, and *(d)* the submaxillary salivary gland were higher in esterolytic activity than those of *(e)* the tongue, region of the fungiform papillae, *(f)* the sublingual salivary gland, *(g)* the parotid salivary gland, and *(h)* the esophagus, portion distal to the pharyngeal end. Tissues *a*, *b*, and *c* of day-old calves were more active than those of older calves and mature cattle. The activity of the submaxillary salivary gland changed very little with age.

Saliva collected from the general area at the base of the tongue was extremely high in esterolytic activity, thus establishing this area as one of secretion. Saliva collected from areas of the oral cavity where the ducts from the parotid, sublingual, and submaxillary salivary glands empty exhibited either little or no activity, thus indicating that the esterase associated with the tissue of these glands was primarily intracellular.

The substrate specificity of the esterase found in saliva obtained at the base of the tongue was studied by employing various fatty acid esters of beta naphthol as substrates. The rate of hydrolysis was highest for the butyrate ester and, with the exception of acetate, decreased as the chain length of the fatty acid increased. The acetate ester was hydrolyzed at approximately the same rate as the laurate ester.

P85. The use of antipyrine in aureomycin-fed dairy calves. D. L. MACFADDEN and C. R. RICHARDS, Univ. of Delaware, Newark.

Ten Holstein calves were paired according to sex and placed in 2 groups: Grp. I, control; and Grp. II, experimental. The calves received colostrum milk the first 5 days, after which they were placed in individual pens and given the following ration for 16 wk.: a milk starter at the rate of 10% of the body weight for 5 wk., grain free choice until they reached a maximum intake of 4 lb. per day, and hay ad lib. Grp. II received 50 mg. of aureomycin (Aurofac 2A) per day for the entire period. At 1, 4, 8, 12, and 16 wk. a dye, antipyrine, was injected intravenously into all calves in an effort to determine the % of body water.

In Grp. II, the increase in body wt., ht. at withers, length from withers to pinbones, and consumption of hay and grain were significantly greater; circumference of the paunch was consistently greater, approaching significance. The incidence of scouring was significantly less in the experimental group.

The mean percentages of body water for Grps. I and II as determined by the use of antipyrine were:

1st wk.—	68.02 ± 1.19;	67.56 ± 2.87
4th wk.—	69.75 ± 5.28;	68.30 ± 0.84
8th wk.—	61.10 ± 3.09;	63.69 ± 4.03
12th wk.—	62.02 ± 4.00;	65.23 ± 3.43
16th wk.—	73.72 ± 15.62;	76.42 ± 15.78

Per cent of body water increased in both groups from 8 to 16 wk.

P86. Effect of chlortetracycline (aureomycin) on young dairy calves in a new environment. F. T. LANDAGORA, L. L. RUSOFF, and R. M. CROWN, Louisiana State Univ., Baton Rouge.

It has been reported that antibiotics are of no value for poultry and animals raised in a new "uncontaminated" environment. This experiment was conducted in a newly constructed calf barn. Animals were kept in individual pens on a cement floor covered with fresh shavings. Thirty new-born Jersey and Holstein male calves were fed colostrum for the first 3 days and then transferred to the new barn. They were divided into 3 groups at random according to breed. Grp. I served as the control, Grp. II received 50 mg. chlortetracycline daily, and Grp. III was administered intramuscularly 400 mg. chlortetracycline in sesame oil weekly. All calves were fed whole milk up to 30 days of age. Good quality hay and a plant protein calf starter were fed at 7 days of age. The calf starter for Grp. II was supplemented with ½% Aurofac 2A.

Scours occurred mainly in the calves in the control group and to a very limited extent in the calves receiving parenterally administered chlortetracycline. At the end of 12 wk. the antibiotic-supplemented Jersey and Holstein calves showed an increase in wt. over the controls by approximately 25% and 15%, respectively. These values were found to be statistically significant at $P < 0.05$. Increased feed efficiency also was found for the antibiotic groups of calves. Leucocyte counts of the blood of antibiotic and control calves showed no significant differences.

P87. Interaction of para amino salycilic acid and aureomycin in the feed of young calves. J. M. WING, Florida Agr. Expt. Sta., Gainesville.

Twenty-five new-born calves of the Jersey and Guernsey breeds were allotted to 5 dietary groups which were comparable with respect to breed, sex, body wt., and ht. at withers. All calves were confined to individual pens and fed colostrum until 4 days of age, whole milk

through 21 days, and reconstituted skimmilk through 60 days, the end of the experimental period.

Supplementing separately 45 mg. per 100 lb. of body wt. daily of crystallin aureomycin (Grp. 1) and 90 mg. P.A.S.A. (Grp. 2) resulted in respective group-mean gains in body wt. of 53 and 51 lb. In combination, the 2 supplements apparently were antagonistic, since subjects of Grp. 3 (which received both) gained an av. of only 43 lb. Reduction of the aureomycin to 4.5 mg. in combination with 90 mg. P.A.S.A. (Grp. 4) resulted in an av. gain of 42 lb. These changes in body wt. compare to a group-mean gain of 39 lb. exhibited by control calves (Grp. 5).

P88. Effect of feeding high levels of chlortetracycline (aureomycin) and tetracycline (achromycin) to newborn calves. L. L. Rusoff, E. J. Stone, A. H. Cummings, and J. B. Frye, Jr., Louisiana State Univ., Baton Rouge.

Two hundred fifty to 1,000 mg. of chlortetracycline and 500 mg. tetracycline per day were fed to newborn Jersey and Holstein calves for the first 3 days. All calves received colostrum during this period. Whole milk was fed from 4 days up to 21 days at the rate of 10% of body wt. and reconstituted skimmilk (1:9) for 35 days. Good quality hay and a plant-protein calf starter was fed to half the calves. The others received the same starter supplemented with ½% Aurofac 2A. Approximately 50 calves were divided at random into 5 groups according to breed and sex. Grp. I served as the control; Grps. II, III, and IV received 250, 500, and 1,000 mg. chlortetracycline, respectively; Grp. V received 500 mg. tetracycline.

Results indicate that high level feeding of antibiotics is of advantage in controlling scours during early life but is of no advantage in stimulating growth. Nutritional levels of chlortetracycline in the ration stimulated growth by approximately 20% at 16 wk. of age.

P89. A comparison of feeding methods as they affect veal production and carcass quality. R. P. Niedermeier, N. N. Allen, and R. W. Bray, Univ. of Wisconsin, Madison.

Male Holstein and Brown Swiss calves were started on experiment after being with their dams 3 days and were slaughtered when 6 wk. old. Liberal whole milk feeding with and without iron supplement was compared to limited whole milk-hay-starter and to liberal milk replacer-hay-starter feeding. All calves were fed 30 mg. aureomycin daily. Milk replacer calves received 25,000 I.U. of vitamin A in addition. To date, 32 calves have been processed. Liberal whole milk calves made an av. daily gain of 2.44 lb. with 8.75 lb. of milk per lb. of gain. Limited whole milk calves averaged 1.50 lb. daily gain, with 6.42 lb. milk per lb. gain. Milk replacer calves averaged 1.38 lb. daily gain and consumed 11.8 lb. milk replacer per lb. of gain. Carcass grades ranged from top choice for the liberal whole milk calves to top utility for milk replacer calves. Color of meat was influenced by iron and copper supplement and by hay and grain feeding. Slaughter data include yield, color, grade, and weights of liver, kidney fat, thymus, and hide. Cooking and taste panel tests are being run. Approximately 40% of the total stomach capacity was in the rumen-reticulum of liberal whole milk calves as compared to 60% for those receiving hay and starter.

P90. Results obtained through the use of frozen bovine semen in field trials.[1] J. W. Snyder, W. D. Rutz, and G. B. Marion, Kansas State College, Manhattan.

Semen from 11 bulls was frozen and used to inseminate all first-service cows in 8 Kansas counties from April 15 through June 30, 1954. Ejaculates which were estimated to have motile spermatozoa of 60% or more and a density of 80×10^7 spermatozoa per ml. or more were extended with a 1:1 yolk–citrate solution so the extended semen would contain between 20 and 40 million motile spermatozoa per ml. Both prefreezing and freezing procedures were essentially as described by Graham and Marion. The semen used in the field trials had an estimated motility of 40% or more after freezing.

The storage period for the semen used varied from 10 to 140 days. Immediately prior to insemination the semen was thawed at 5° C. A 60-90 day nonreturn rate of 67% was obtained for 904 first-service cows. When fresh semen from the same bulls was used during the same period a 60-90-day nonreturn rate of 64% was obtained for 3,990 first-service cows. Semen not used in the first trial was further stored for 122 days. It was then returned to 7 of the same counties and used a varying number of days during November. A 60-90 day nonreturn rate of 58% was obtained for 221 first-service cows. When fresh semen from these bulls was used during November a 60-90-day nonreturn rate of 64% was obtained for 1,870 first-service cows.

[1] This study was supported in part by the National Assoc. of Artificial Breeders, Columbia, Mo.

P91. Equipment for technicians' storage and field handling of frozen semen. H. H. Brugman and M. E.. Poore, Univ. of Maine, Orono.

Many artificial breeding cooperatives have refrained from utilizing the potentialities of frozen semen because of the high consumption and cost of solid CO_2, of technicians' storage facilities, and of field handling equipment.

Storage cabinets of 4 cu. ft. capacity were developed with space for 450 ampules and 175 lb. of CO_2, 1,250 ampules and 135 lb. CO_2, or 2,500 ampules and 100 lb. CO_2. The consumption of CO_2 varied from 7½ to 10 lb. per day, depending on whether the CO_2 was crushed or

in blocks. Temperatures maintained varied from –70° to –76° C., depending on whether or not solid CO_2 was added to the alcohol storage bath.

Temperatures indicated were maintained for as long as 2 wk. without adding CO_2. Ampule holders were constructed to facilitate storage of 1,250 ampules in a space 10¾″ by 11¾″by 9″. All ampule holders or cartridges are readily identified from the top and can be hooked together for increased central storage capacity.

P92. Variation in survival of bovine spermatozoa when stored at subzero temperatures as affected by differences in breeds, bulls, and ejaculates. M. E. POORE and H. H. BRUGMAN, Univ. of Maine, Orono.

The potentially widespread use of frozen semen has made it desirable to obtain information relative to the variation in survival of bovine spermatozoa as affected by breeds, bulls, and ejaculates when stored at subzero temperatures (–76° C.). Four New England artificial breeding cooperatives shipped semen from 40 bulls of 5 dairy breeds, every 2 wk. for 10 mo. The extender formulated by Dunn *et al.* was used. Technique for glycerolating followed the method described by Polge *et al.* The rate of temperature decline was 0.8° C. per min. from +5° to –15° C. and 3° to 6° C. per min. until –76° C. was obtained. Dilution rates of 1:40 were used. Percentage motility estimates were made on samples thawed at 5° and 38° C. immediately after freezing and after 7 days storage at –76° C.

The data indicate that there is a difference between breeds, between bulls within breeds, and between ejaculates within bulls of bovine spermatozoa survival at subzero storage temperature. However, differences are small, and spermatozoa which survive subzero temperatures to a high degree will do so within a narrow deviation from the mean.

P93. The effect of glycerol equilibration time on the freezing of bovine spermatozoa in egg yolk–citrate and skimmilk semen diluters. G. D. O'DELL and VICTOR HURST, Clemson Agr. College, Clemson, S. C.

An experiment was designed to compare the effects of zero and 18-hr. glycerol equilibration periods on freezing bovine spermatozoa. Skimmilk and egg yolk–citrate diluters were used. The yolk–citrate diluter was composed of 20% egg yolk and 80% 0.1 *M* sodium citrate. Glycerol was added to the diluters in 5 steps at 6-min. intervals to give an 8% glycerol level. Final dilution of semen was 1:50. Freezing from 5° to –15° C. was carried on at a rate of 0.8° C. per min. Freezing from –15° to –79° C. was carried out as rapidly as possible. All freezing was done in alcohol with Dry Ice.

Results were based upon the % recovery of sperm cells 48 hr. and 10 days after freezing. In both types of diluters sperm cells showed statistically higher recovery rates when equilibrated in glycerol for 0 hr. than when equilibrated 18 hr.

P94. The influence of added lipoprotein on the freezing of bovine spermatozoa. G. BIALY[1] and E. A. HESS, The Ohio State Univ., Columbus.

Lipoproteins were isolated from egg yolk, by the method described by Kamschmidt and Chargaff, and added at various levels to 47 diluted semen samples. In a split ejaculate trial, samples were frozen with 0, 2.5, 5, and 10% lipoproteins and stored in a mechanical refrigerator at –96° C. Evaluations of the samples for motility and % of live cells were made immediately after freezing, after 2 days, 9 days, 16 days, and 23 days.

The results indicate that the addition of 5% lipoproteins to the regular diluted semen is near the optimum level for maximum improvement in semen quality. Comparison of the control and the 5% lipoprotein samples for motility and live cells indicated the superiority of the samples to which lipoproteins were added.

[1] Now at Univ. of Wisconsin, Madison.

P95. The relation of carotene intake to the carotene and vitamin A values of the plasma, liver, and milk fat of dairy cattle. I. R. JONES, P. H. WESWIG, J. F. BONE, and B. F. MAGILL, Oregon State College, Corvallis.

Studies were continued on the vitamin A and carotene content of plasma, milk fat, and liver biopsy samples of dairy cattle. Eight animals were fed high carotene rations, including grass silage and alfalfa hay in winter and irrigated pasture in summer. Four animals (Grp. I) and 3 animals (Grp. II) received low carotene rations including barley-ryegrass hay (2.3 p.p.m. carotene) supplemented with carotene in carrot oil to 130γ and 390γ carotene, respectively, per kg. of body wt.

With the high carotene ration, plasma carotene and milk fat carotene and vitamin A reflected carotene intake, but plasma vitamin A was extremely variable. Liver carotene and vitamin A values were not consistent with each other or with plasma or milk fat values. Calving did not appear to alter liver stores of vitamin A. The seasonal variation of about 75 liver vitamin A values ranged from about 50γ to over 200γ per g. of liver, with highest values in October or November.

The carotene and vitamin A values of plasma, milk fat, and liver averaged higher for Grp. II than for Grp. I. The av. liver vitamin A per g. from 32 biopsies in Grp. I was 12.4γ (range 4.9-26.1γ) compared to 18.7γ (range 7.2-34.5γ) for 24 biopsies in Grp. II. These values indicate that recommended carotene allowances result in minimal storage.

P96. Effect of previous ration on lactation response to substitution of corn for alfalfa according to NE and TDN systems of estimation. R. TEICHMAN, H. D. EATON, G. BEALL, and R. E. JOHNSON, Conn. Agr. Expt. Sta., Storrs; H. L. LUCAS, JR., N. C. State College, Raleigh; and L. A. MOORE, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

The lactation response to the substitution of corn for 10 lb. of alfalfa on the basis of either TDN or NE was studied, in a double reversal trial, with 24 cows fed equivalent NE/100 lb. live wt. from a previous ration of either 100% alfalfa or 80% alfalfa plus 20% corn. Basing the substitution on the TDN system resulted in greater av. daily intake of lb. of TDN and therms of NE than when the NE system was used. Substitution of corn for alfalfa on the basis of TDN resulted in 0.74 lb. FCM more than when the NE system was used, but the magnitude of difference depended on the previous ration. When the substitution was based on the NE system, cows previously fed the 80% alfalfa plus 20% corn ration produced 0.46 lb. FCM less than the 100% alfalfa group. When the substitution was based on the TDN system, cows previously fed the 80% alfalfa plus 20% corn ration produced 2.17 lb. FCM less than the 100% alfalfa group. Cows previously fed the 80% alfalfa plus 20% corn ration produced, over all periods, 1.32 lb. FCM less than the 100% alfalfa group.

P97. Urea versus cottonseed meal as the chief source of protein for young dairy steers, with a study of methods used to determine the efficiency of urea utilization. I. LEVY and J. D. DONKER, Univ. of Georgia, Athens.

The rate and efficiency of gain of Holstein steers 3¼ to 7½ mo. old were compared on 3 rations. Ration 1 was ground straw, ground snapped corn, molasses, vitamins A and D, and minerals (8% crude protein). Crude protein was increased to 12% by urea (Ration 2) or cottonseed meal (Ration 3). The animals were paired and randomly placed in 2 groups of 5 according to age, wt., and rate of gain during Period I. There were 4 periods: Period I—8 wk. of ration 3 to both groups; Period II—12 wk. of ration 1 to Grp. 1 and ration 2 to Grp. 2; Period III—12 wk. of ration 1 to Grp. 2 and ration 2 to Grp. 1; Period IV—7 wk. of ration 3 to both groups.

Total daily urine collections were periodically measured and analyzed for urea, ammonia, and creatinine. A digestion trial, comparing an index method with the conventional method, was run. The av. daily gain and ration consumed per lb. of gain was: ration 1—0.94 and 14.1 lb.; ration 2—1.58 and 9.21 lb.; ration 3—2.34 and 7.63 lb.

P98. Comparison of cottonseed meal, molasses containing urea, and ammoniated molasses as protein supplements for dairy heifers. W. A. KING, G. D. O'DELL, J. P. LAMASTER, and D. B. RODERICK, Clemson College, Clemson, S. C.

Molasses containing 5% urea and ammoniated molasses were compared in a 112-day feeding trial to cottonseed meal as sources of protein with 3 groups of heifers containing 25 each. The groups, rations, and amounts consumed per heifer were as follows: Grp. 1 (control) 44.9 lb. corn silage, 2 lb. cottonseed meal and 3 lb. molasses; Grp. 2—44.1 lb. corn silage, 1 lb. cottonseed meal and 3 lb. molasses containing 5% urea; Grp. 3—43.3 lb. corn silage, 1 lb. cottonseed meal and 3 lb. ammoniated molasses (14.7% crude protein equivalent). Trace mineralized salt and dicalcium phosphate were available free-choice. The av. daily gains were: Grp. 1— 1.71 lb., Grp. 2—1.44 lb., and Grp. 3—0.92 lb., for the first 84 days. During the last 28 days Grp. 3 was fed an additional lb. of cottonseed meal per heifer daily and the gains averaged 1.90 lb.

Digestion and nitrogen balance studies show equally good utilization of the feeds between Grps. 1 and 2. The daily nitrogen balance averaged +10.7 g. for Grp. 1 and +15.3 g. for Grp. 2.

Palatability studies showed that the cattle preferred the molasses in order of blackstrap molasses, molasses with 5% urea, and ammoniated molasses. There were only small differences between the first two.

P99. Variability in the quality of untreated high moisture silages. C. H. GORDON, C. G. MELIN, H. M. IRVIN, and H. G. WISEMAN, Dairy Husbandry Research Branch, and L. E. CAMPBELL, Agr. Eng. Research Branch, USDA, Beltsville, Md.

First cutting forage, principally Ladino clover and orchard grass, was harvested in 1953 and 1954 and stored without wilting or preservatives in 10′ × 25′ silos. Excepting sugar (5.4 and 8.8% on a dry matter basis, respectively, in 1953 and 1954) the original forages were of similar composition. Dry matter percentages were 19.4 and 18.4, respectively. Composition changes, nutrient losses, digestibility, and feeding values for dairy cows were determined for each silage. Although prepared by procedures of the same general description, the 1954 crop silage was superior in quality, particularly with respect to pH, ammoniacal nitrogen, lactic acid, and butyric acid. These differences were so marked that the addition of preservatives to a companion silage was shown to have a distinct advantage in 1953 but little or no advantage in 1954. These results emphasize the importance of control silage when evaluating the net effect of any silage treatment. The need for additional fundamental information regarding the specific biological interrelationships existing in the control silage is apparent.

P100. Effects of sodium metabisulfite and degree of compaction on composition of legume-grass silage in miniature silos. R. S. ALLEN and RUTH M. WARD, Iowa State College, Ames.

Twelve miniature concrete silos (2 ft. in diameter, 6 ft. high) were employed. Treatments constituted a 3 × 2 factorial design with 3 degrees of compaction (light, medium, and heavy) and the absence or presence of sodium metabisulfite (8 lb/ton added at the field harvester) in nonwilted legume-bromegrass forage. Ammonia nitrogen, carotene, total acidity, acetic acid, butyric acid, lactic acid, and pH determinations were made on the forage as ensiled and on the silages when the silos were opened after 5-6 mo.

The pH values tended to decrease with increased packing, with the metabisulfite-treated silages exhibiting consistently lower figures. Ammonia nitrogen and butyric acid increased with increase in packing in the nonpreserved silages, whereas an opposite trend was observed with the preserved silages. Lactic acid, total acidity, and carotene values were maximum with medium compaction of the metabisulfite-preserved silages. The former was higher in the preserved silages regardless of degree of packing. Carotene levels were somewhat higher in the preserved silages, whereas the total acidity values generally were higher in the nonpreserved silages. The acetic acid contents were considerably higher in the metabisulfite-preserved silages.

P101. The effect of herbicides on in vitro cellulose digestion by rumen microorganisms.[1] R. G. JENSEN and C. P. MERILAN, Univ. of Missouri, Columbia.

Samples of rumen contents obtained from fistulated cattle and sheep were incubated for 7 hr. under anaerobic conditions in Warburg manometric equipment. Cellulose digestion, as indicated by increased gas release compared to the basal rate, began after approximately 4 hr. of incubation.

The addition of 2,4-D to the reaction vessels in concentrations ranging from 0.5 to 500γ per ml. of rumen fluid did not cause a reduction in fermentation of the cellulose substrate. Cellulose digestion was slightly reduced by the addition of 500γ of 2,4,5-T per ml. of rumen fluid.

[1] Contribution from the Missouri Agr. Expt. Sta., Journal Series No. 1516. Approved by the director.

P102. The influence of the lignin content of long hay on the passage of nutrients through the rumen. O. T. STALLCUP, J. L. CASON, and B. J. WALKER, Arkansas Agr. Expt. Sta., Fayetteville.

Sericea lespedeza, prairie, Korean lespedeza Lot 1, Korean lespedeza Lot 2, and Korean lespedeza Lot 3 hays containing 32.96, 27.32, 19.96, 17.03, and 15.46% lignin, respectively, were fed long to 2 fistulated steers. The rumen contents were removed, sampled, and returned before feeding. The contents were again removed, sampled, and returned at 6 and 12 hr. after feeding. There was a highly significant negative correlation ($P < 0.01$) between the lignin content of the hay and the % removal of lignin and ash within 12 hr. Since lignin has been used as a tracer for forage digestibility studies, it appears that the digestibility of the hays is likewise negatively correlated to the lignin content of the hay. The deviation from zero of the regression of the lignin content of the hay ingested on the % removal of lignin and ash from the rumen was significant ($P < 0.05$).

P103. The effect of light on optical density of extracts of fecal pigments in digestibility studies. E. A. KANE and W. C. JACOBSON, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Digestibility data obtained by plant pigment methods have been brought into question by the finding of Lancaster and Bartrum that acetone extracts of feces are remarkably sensitive to exposure to light. At the same time acetone extracts of forage appear to be stable to light. This laboratory has confirmed the results of Lancaster and Bartrum. Extracts of feces from animals on various roughages and from calves on synthetic diets without roughage were examined. Increases in optical density up to 39% have been found when acetone extracts of feces were exposed to light of measured intensity for a period of 1 hr. Results of this study suggest that pigments originating in the digestive tract may be involved in contributing to the increase in optical density. The effect of the increase in optical density of fecal extracts on digestibility data needs further study.

P104. Digestibility of certain carbohydrate fractions of forages by ruminants. R. E. ELY and L. A. MOORE, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Wheat straw, alfalfa silage, corn silage, and 7 hays were fed as the entire ration to dairy cows. The lignin ratio technique was used to calculate the digestibility coefficients of carbohydrate fractions determined or calculated by the following methods: (1) crude fiber and nitrogen-free extract, (2) holocellulose (cellulose and hemicellulose), and other carbohydrates, and (3) total carbohydrates.

Holocellulose was more digestible than the crude fiber fraction in all of the feeds studied. Crude fiber contains most of the indigestible lignin which is largely removed from the holocellulose fraction. The digestion coefficients of holocellulose ranged from 49.5 to 82.5.

Wheat straw was the only forage that did not contain highly digestible carbohydrates. The holocellulose fraction accounted for most of the carbohydrates in wheat straw. The calculated

total carbohydrate content was more digestible than the holocellulose fraction in all of the forages except wheat straw. The calculated TDN content of the ten forages ranged from 36.0 to 70.5%.

P105. Value of identical twin cattle in digestibility studies. CLARENCE CHESNUTT, JR., and I. R. JONES, Oregon State College, Corvallis.

A series of 8 standard digestion trials have been conducted in conjunction with roughage studies. Six of the trials were made on grass or legume-grass silages with or without preservatives and 2 were made on hays. Used in these studies were 4 sets of identical twin and 3 pairs of single-birth females, of which the latter and 2 sets of the twins were lactating. The other 2 sets of twins were in early pregnancy.

The 8 twin and 6 single-birth animals were used in combination to yield within-pair and between-animal comparisons for TDN, protein, ether extract, fiber, nitrogen-free-extract, and dry matter. By analysis of variance, the twin efficiency values using the Bonnier and Hansson method were: TDN, 14; protein, 8; ether extract, 3; fiber, 16; nitrogen-free extract, 11; and dry matter, 17. The results of this study indicate the superiority of identical twin cattle in studying the comparative digestibility of feedstuffs.

P106. Effect of feeding various levels of fluorine, calcium, phosphorus, and grain to dairy heifers from four months to thirty-two months of age. G. E. STODDARD, J. L. SHUPE, M. L. MINER, L. E. HARRIS, D. A. GREENWOOD, W. BINNS, G. Q. BATEMAN, H. NIELSON, and D. STRONG, Utah State Agr. College, Logan.

Thirty-two grade Holstein heifers were assigned to treatments of 4 levels of fluorine (basal, 25, 50, and 100 p.p.m. based on hay intake), 2 levels of defluorinated phosphate (1 and 3% of grain mix), and 2 rates of grain feeding (2 and 4 lb. daily before calving and 1 lb. daily for each 0.18 and 0.14 lb. of butterfat produced daily).

Fluorine and phosphate level did not influence hay consumption or total dry matter intake. Heifers on high rate of grain (1:0.14) consumed less hay and total dry matter than those on standard rate (1:0.18). Gain in body wt. and increase in ht. at withers were not affected by fluorine intake, although some trends near the end of this period were questionable. Heifers on high rate of grain gained more than low-grain heifers, but ht. at withers was not affected by rate of grain feeding.

Conception rate was not affected by treatment. Examination every 2 wk. has shown a relationship between fluoride intake and tooth lesions, exostosis and stiffness of heifers. Fluorine content of biopsied rib samples has been closely associated with fluoride intake. Ten-day metabolism trials were run on heifers 68-113 days and 469-509 days after experiment started.

P107. The effect of added calcium and trace elements on mineral balance in dairy cows in early lactation.[1] R. E. MATHER, New Jersey Agr. Expt. Sta., Sussex.

Guernsey and Holstein heifer calves were placed in 4 ration groups at birth and carried into 3rd lactations. Rations were I, control (commercial concentrate); II, control plus 2.5% C. P. calcium carbonate; III, control plus 3.0% calcite flour; IV, control plus 3.0% commercial mineral mixture (calcite flour plus Fe, Cu, Mn, Co, Zn, and I). Sixty-one 10-day balance trials were conducted in 3 lactations, beginning an av. 57 days after calving. Multiple regression estimates of calcium balance per 1,000 lb. cow per day, in grams, for the 4 rations, with the 95% confidence intervals, were -9.0 ± 8.1, -2.8 ± 7.5, -3.4 ± 8.8, and -5.2 ± 7.5 for third lactation Guernseys. First lactation values were more positive by 12.7 g. ($P < 0.05$) than 3rd lactation, and 2nd lactation more positive by 3.6 g. ($P > 0.05$). Holsteins were less negative than Guernsey values above by 1.2 g. ($P > 0.05$). A cumulative effect of trace elements was not shown by 3rd lactation data. Calcium intake was positively correlated with calcium balance ($P < 0.05$).

[1] This work was supported in part by the Limestone Products Corp. of America, Newton, N. J.

P108. Trace mineral deficiencies in cattle resulting from heavy fertilization of the soil. H. A. KEENER, F. E. ALLEN, and H. A. DAVIS, New Hampshire Agr. Expt. Sta., Durham, and K. C. BEESON and E. J. THACKER, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y.

The chemical composition of timothy, bromegrass, and ladino clover grown on heavily fertilized soil has been determined for the past 5 yr. Such forages often have been low in iron, cobalt, copper, and magnesium and relatively low in manganese. Calcium and phosphorus levels have been normal.

The nutritive value of such forages is being studied with dairy cattle that were started on experiment as young calves. They have been fed either timothy or ladino clover–bromegrass hay and a low mineral concentrate mixture. Deficiency symptoms observed were poor growth, rough coats, anemia, sagging of spinal column behind shoulders, an ataxia of hind legs (timothy fed calves only), loss of tips of ears, and broken bones. After freshening, most animals lost wt. rapidly, milk production was low, and appetites have been poor and often depraved. All animals have indicated various signs of nervousness. During the past 2 yr. many of the calves produced by cows on the experiment have shown varying degrees of goiter. This has not been explained on the basis of a low iodine content of the forage.

P109. The effect of pulverized limestone and dicalcium phosphate on the nutritive value of dairy cattle feed. N. F. COLOVOS, H. A. KEENER, and H. A. DAVIS, New Hampshire Agr. Expt. Sta., Durham.

Sixteen individual experiments were carried out with four 18-24 mo. old heifers to determine the effect of pulverized limestone and dicalcium phosphate on digestibility and utilization of the protein and energy of the ration. During the course of the study each animal was on a balance experiment in which the grain portion of the ration was supplemented as follows: no supplement, 2% limestone, 2% dicalcium phosphate, and 2% limestone plus 2% dicalcium phosphate.

The 2% limestone decreased the digestibility and daily balance of both protein and energy. This confirms previous work with silage at this station. The dicalcium phosphate did not have any appreciable effect. The addition of 2% dicalcium phosphate to the 2% limestone supplement minimized the depressing effect of the limestone.

Balance studies to determine the effect of 1% limestone and 1% dicalcium phosphate are now in progress with the same animals and the same rations.

EXTENSION SECTION

E1. Effective techniques in extension teaching. K. F. WARNER, Federal Extension Service, Washington, D. C.

Effective teaching is the transfer of facts, skills, and attitudes so that they will be understood, learned, remembered, and used. Teaching efforts are wasted if the behavior of the learner is not influenced by what is taught.

The effective teacher must: *(a)* Capture attention by helping the listener visualize the facts. *(b)* Keep the listener interested through a change of pace by use of action, visual aids, participation. *(c)* Be sure listeners understand; words and action must convey the ideas you want them to have. *(d)* Help listeners remember. *(e)* Include a planned follow-up. Stimulate people to discuss and talk about the subject afterwards.

E2. Television for dairy extension work. G. H. AXINN, Michigan State College, East Lansing.

Television, a revolution to the communications industry, is but another tool to the extension dairyman. It can save him time, money, and travel. It offers him sight, sound, motion, and immediacy. He can use it to reach people he has never reached before, including consumers of dairy products, as well as milk producers.

Television is growing toward the saturation point in rural American homes. We will have more television stations, and soon most homes will have receivers. Already, about 125 stations are equipped to carry color. Color will make television more real, more exciting, more dramatic, more enjoyable—and, most important, a better teacher!

Tape recording of television and other such innovations which are just around the corner will make this new channel a more flexible, more effective tool for extension dairymen. Integrating it into a well-planned program is your challenge.

E3. The role of dairy extension specialists in farm and home development. J. E. CROSBY, JR., Federal Extension Service, Washington, D. C.

Farm and home development is a superior method of extension teaching. It is a method available to all people, and its use must be considered by all extension workers.

A farm family may consider farm and home development to consist of: *(a)* identifying and analyzing effectively all their major farm and home problems and opportunities, *(b)* assembling and evaluating all available data pertinent to these problems, *(c)* choosing tentative solutions to the problems, *(d)* testing tentative decisions by fitting them into a system of farming and family living, *(e)* acting to put the system finally approved into operation, and *(f)* revising the system whenever advisable.

Most segments of extension can contribute to this process. Dairy specialists can help farm families become aware of the opportunities to secure income through dairy farming. Second, data and subject matter on dairying can be assembled by them. In most cases, county extension agents will discuss these with farm families and teach them to analyze and interpret the facts. Third, county agents will need help on unfamiliar problems. Fourth, translating plans into action will require additional knowledge, increased skills, and organization to provide materials, equipment, and facilities. Finally, dairy specialists can contribute by reorganizing their subject matter to make it apply more directly to specific farm problems.

E4. Using D.H.I.A. records as an effective teaching tool.

A panel consisting of J. D. BURKE, New York *(moderator)*; H. C. GILMORE, Pennsylvania; C. H. PARSONS, Massachusetts; and D. E. VOELKER, Iowa, will discuss methods of using D.H.I.A. records in extension teaching. Methods of analyzing results and examples from the above states will be presented. Attention will be given to D.H.I.A. result studies found useful in group teaching, as well as material that can be used by the specialist, county agricultural agent, or D.H.I.A. supervisor in working with the individual members.

E5. Standardized procedures for the Babcock test for milk. E. O. HERREID, Univ. of Illinois, Urbana.

The need for standardizing testing techniques in dairy herd improvement associations depends

on whether the tests will be used for semi-official or official records, or will be used as a guide to feeding practices and herd productivity.

Fat tests made by the plant and those made by the D.H.I.A. may not agree because: *(a)* The number of tests over a given period is not the same. *(b)* The D.H.I.A. tests are from one day's production, subject to daily fluctuations in fat percentage and milk yield. *(c)* Milk plants usually have better facilities, making it easier to obtain more uniform results.

Since not all techniques in the Babcock test have been standardized, the A.D.S.A. has approved unified procedures, which were published (*J. Dairy Sci.*, **37**: 761. 1954).

E6. Detergent mixtures for testing fresh milk samples. E. O. HERREID, Univ. of Illinois, Urbana.

Detergent mixtures for testing milk are making their third appearance in the dairy industry in the past 50 years. They were first prominently mentioned in the literature in Europe about 1900-1905, then in this country in 1928-1930 at the Illinois and Minnesota experiment stations, and more recently at the Bureau of Dairy Industry in Washington, D. C.

A committee in the A.O.A.C. is evaluating the accuracy and practicability of the B.D.I. test as compared to the Babcock and Mojonnier methods. This is being done in industry, regulatory, and experiment station laboratories.

E7. Comparison of the B.D.I. detergent test and the Babcock test under herd conditions. F. M. GRANT, Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Duplicate samples of the B.D.I. test, if run by an experienced operator, will be the same about as often as with the Babcock test. However, when the duplicates vary, there seems to be a tendency for the B.D.I. tests to vary by larger amounts.

When the averages of duplicates of the B.D.I. test were compared with those of the Babcock test, they agreed within 0.05% in over 2/3 of the comparisons. When the B.D.I. test varied from the Babcock, it was higher about as often as it was lower, and there was no appreciable difference in the amount of variation.

An experienced Babcock tester can quickly learn to operate the B.D.I. test and can complete it in practically the same time as the Babcock test.

E8. Dairy cattle health problems. B. T. SIMMS, Animal Disease and Parasite Research Branch, USDA, Beltsville, Md.

E9. Enlisting, developing, and using 4-H Club leaders. P. A. MILLER, Michigan State College, East Lansing.

In working with the volunteer leader in 4-H Club work the challenge for leadership is commonly overlooked. Construing the work of the volunteer leader as a challenge means relating it to both the person and community dimensions of the leader's life. Another overlooked skill is that of adequately providing recognition to the volunteer. One of the potential weaknesses of the 4-H Club programs is that of standardizing the rewards to its leaders.

A third opportunity sometimes missed by professional workers cooperating with the volunteer has to do with the use of variety. Do the 4-H Club leaders, after a second or third year, begin to lead a predictable 4-H career? To become slaves to an unvarying set of procedures will result in loss of members. Sometimes, also, professional workers in 4-H Club work fail to encourage the leaders to make decisions.

Finally, the volunteer leaders must understand that 4-H Club work is not just another activity but an enterprise that fills a vital need in the community. There is some doubt that the leadership in 4-H Club work has done as good a public relations job in the home community as it has on the state level.

Panel discussion.

Panel members:

GEORGE HYATT, *moderator*, North Carolina State College
IRVING WYETHE, Michigan County Agent
A Michigan local leader (to be announced later)
R. D. STEWART, American Guernsey Cattle Club
NEVELS PEARSON, Asst. Michigan State 4-H Club Leader
J. D. GEORGE, North Carolina State College
RALPH BONEWITZ, Kansas State College

THE USE OF 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE AS A TEST FOR ANTIBIOTIC SUBSTANCES IN MILK[1]

C. E. NEAL AND H. E. CALBERT
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The importance of the presence of antibiotics in raw milk has been discussed by various workers *(1, 4, 6)*. The need for a simple test to determine the presence of starter inhibitory substances in raw milk has been evident since the introduction of antibiotics in the treatment of mastitis. A test that could be easily applied to individual patron samples to detect the presence of inhibitory substances would have important application. This is true particularly when the milk is to be used in the manufacture of cheese or other fermented dairy products.

The lack of development of lactic acid by dairy starters has been used to determine the presence of inhibitory substances in milk *(4, 6)*. The paper disk assay method also has found application for this purpose. A modification of this method, employing *Bacillus subtilis* spores seeded on whey agar, has been reported by Churchill and Frank *(2)*. Friedmann and Epstein *(3)* have adapted the resazurin test for the assay of antibiotics in milk using a strain of *Streptococcus cremoris*. The methylene blue reduction test has been employed by Schipper and Petersen *(5)* as a measure of inhibition of a test organism against aureomycin with sterile milk. Many of these methods do not lend themselves to routine testing procedures without the need for specialized equipment and extensive training of the laboratory worker.

Wallhausser *(7)* has employed 2,3,5-triphenyltetrazolium chloride to detect inhibition of bacterial growth when testing for antibiotics in body fluids. His method is essentially a plate assay using *B. subtilis* or *B. mycoides* as a test organism. The presence of growth is determined by the formation of a red color when no inhibition has occurred.

The object of the following investigation was to develop a simple and effective test employing 2,3,5-triphenyltetrazolium chloride as an indicator for inhibition of a test organism in the presence of antibiotic substances in milk.

METHODS

In the presence of metabolizing bacterial cells, 2,3,5-triphenyltetrazolium chloride (TTC) is converted to formazane. This conversion in a milk medium is noted by a change from the characteristic color of the milk to a reddish pink.

S. thermophilus and a commercial lactic starter[2] were used as test cultures. The cultures were carried on a sterile skimmilk medium (10% reconstituted nonfat dry milk solids, free from inhibitory substances). The cultures were

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[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Obtained from the General Biochemicals Inc., Laboratory Park, Chagrin Falls, Ohio.

transferred daily and used for inoculum when 12 to 14 hours old. The *S. thermophilus* culture was incubated at 37° C. and the lactic starter at 22° C. Inoculum was prepared for use in all determinations by diluting a culture 1:1 with sterile skimmilk to facilitate ease of pipetting.

For each series of samples, various quantities of an antibiotic standard were pipetted into sterile test tubes, then raw milk was added to make a total volume of 9 ml. The samples were then pasteurized in a water bath at 80° C. for 5 minutes, removed, and quickly cooled to below 37° C. Each sample was then inoculated with 1 ml. of diluted 1:1 inoculum to give a total volume of 10 ml. per tube. The tubes were stoppered with sterile rubber stoppers. Just prior to incubation, the tubes were inverted twice to mix the contents. The samples were incubated for 2 hours in a temperature-controlled water bath at 37° C. After this incubation period the samples were removed and 0.3 ml. of a 1:25 solution of 2,3,5-triphenyltetrazolium chloride[3] (prepared with distilled water and stored under refrigeration, 7° C.) was added to each tube. Again the tubes were inverted twice to mix the solution with the sample and then returned to the incubator for an additional period of 30 minutes.

For each series of samples, two control tubes were prepared in the same manner and subjected to the same treatment as all other samples with the exception that no antibiotic was added to these controls. For comparative purposes, a 14-hour commercial lactic starter was used as a test culture in the same manner as the *S. thermophilus* culture.

The samples were observed at the end of the 30-minute period. Samples that were white or had various shades of pink lighter in color than the control samples were considered to contain substances that are inhibitory to the growth of the test organism. When variation in color occurred, the less intense the pink color the greater was the amount of inhibitory substances present. When there was no detectable color change, it was considered that complete inhibition had occurred.

RESULTS

When four of the more common antibiotics in use, namely, penicillin, aureomycin, terramycin, and streptomycin, were added to various samples of raw milk, inhibition of *S. thermophilus* and a commercial lactic starter could be detected in the presence of 2,3,5-triphenyltetrazolium chloride at relatively low concentrations. As shown in Table 1, the *S. thermophilus* culture had a greater sensitivity against all the antibiotics except streptomycin under the test conditions than did the commercial lactic culture. With the *S. thermophilus* culture the characteristic pink color was not produced at concentrations in the milk of 0.04 unit of penicillin per milliliter, 0.2 γ per milliliter of aureomycin, and 0.25 γ per milliliter of terramycin. With streptomycin at a concentration of 4.0 γ per milliliter in the milk a slight pink color was observed. Inhibition of the commer-

[3] 2,3,5-triphenyltetrazolium chloride was obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 1

The concentration of antibiotics detectable in raw milk using the 2,3,5-triphenyltetrazolium chloride test

Antibiotic	*S. thermophilus*	Commercial lactic starter
Penicillin[a] *(units per ml.)*		
0.03	+++	+++
0.04	−	+++
0.05	−	+++
0.10	−	+++
0.20	−	++
0.30	−	−
Aureomycin[b] *(γ per ml.)*		
0.05	+++	+++
0.10	++	++
0.15	+	++
0.20	−	++
0.25	−	+
0.30	−	−
Terramycin[c] *(γ per ml.)*		
0.10	+++	+++
0.15	++	+++
0.20	+	+++
0.25	−	+++
0.30	−	+++
0.60	−	++
1.00	−	+
Streptomycin[d] *(γ per ml.)*		
0.30	+++	+++
0.40	+++	++
0.50	+++	+
0.60	+++	−
3.00	+++	−
3.40	+++	−
3.80	+++	−
4.00	+	−
4.20	−	−
Control	+++	+++

The intensity of color formation is indicated by plus (+) signs with +++ indicating that no inhibition was expressed; ++ and + show increasing degrees of inhibition with a minus (−) indicating no color and complete inhibition.

[a] Penicillin G-sodium, Merck and Co.

[b] Chlortetracycline HCl, Lederle Laboratories.

[c] Calcium dioxytetracycline, Chas. Pfizer and Co.

[d] Dihydrostreptomycin sulfate, Merck and Co.

cial lactic starter was noted at the following concentrations in the milk: penicillin 0.3 unit, aureomycin 0.3 γ, terramycin a slight pink color at 1.0 γ, and the streptomycin at 0.6 γ per milliliter.

DISCUSSION

The use of 2,3,5-triphenyltetrazolium chloride can prove helpful in testing for antibiotics in milk in conjunction with organisms that are sensitive to antibiotics. As the test has been outlined, a person familiar with simple laboratory

techniques should have no difficulty in using it to detect starter inhibitory substances in milk.

It is essential that the inoculum be measured accurately to assure that there are equivalent amounts of inoculum in each sample. Another critical step of the determination is the observation of the samples at 30 minutes after addition of 0.3 ml. of TTC reagent. In the presence of streptomycin and terramycin a longer period of incubation of the sample will decrease the ability of the test to detect low concentrations of these antibiotics.

The use of *S. thermophilus* as a test organism does not detect concentrations of streptomycin at levels as low as does the use of the commercial lactic starter. However, this limitation should not seriously impair the test, as streptomycin is often used in conjunction with other antibiotics in the treatment of mastitis. When the test is applied to the milk supply from individual patrons, the presence of antibiotics in the milk can be detected at concentrations below which the antibiotics would have no commercial significance in the manufacture of fermented dairy products.

It is believed that the sensitivity of this test is dependent upon the age and relative size of the inoculum as well as the duration of incubation before addition of the TTC reagent. In performing this test an attempt has been made to strike a balance between these variables to give a sensitive test in the shortest length of time. Modification of these methods, no doubt, will give other levels of sensitivity.

In testing raw milk samples it is necessary that they first be pasteurized to destroy any growing bacteria, for if living bacterial cells are not destroyed they may not be inhibited by the antibiotics, thus giving erroneous results. The possibility of bacteriophage affecting the test organism is minimized in the test by pasteurization at 80° C. for 5 minutes.

When establishing the concentration levels of the various antibiotic substances that could be detected by the TTC test, the antibiotic standard was added to the raw milk prior to pasteurization. As the test was performed on the sample after heating, the detectable concentrations reported are those that existed in raw milk, not the concentration after the heat treatment. The test, therefore, allows for the possible inactivation of the antibiotic when subjected to the heat treatment.

In the application of this test to raw milk samples, the test does not detect inhibitory substances that are heat-labile at the temperature applied to the milk. The presence of germicidal substances such as quaternary ammonium compounds and other compounds used in the treatment of mastitis will also influence the test if in high enough concentration. Therefore, the test is not specific for antibiotics; instead, it is a general test for inhibitory substances that will affect the test culture. Data have been presented on the detectable concentrations of antibiotics because it is believed that these are the compounds often encountered as inhibiting lactic starters.

The use of this test has been found effective in the detection of inhibitory substances in milk from individual cows under treatment for mastitis. The test

likewise has been employed in testing the mixed milk of individual herds to detect inhibitory substances in milk used for manufacture of cheese.

SUMMARY

A simple test for detection of inhibitory substances in milk is outlined. The test is based on the conversion of 2,3,5-triphenyltetrazolium chloride (TTC) to formazane. The reaction is characterized by a color change from the leucoform to red in the presence of growing bacterial cells. This conversion is inhibited by antibiotics at low levels of concentration. When *S. thermophilus* is used as a test organism, penicillin at 0.04 unit, aureomycin at 0.2 γ, terramycin at 0.25 γ, and streptomycin at 4.0 γ per milliliter can be detected in raw milk. The total time for the test is 2½ hours.

ACKNOWLEDGMENTS

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IMPROVEMENTS IN A METHOD FOR DETERMINATION OF THE COLOR OF MILK AND MILK PRODUCTS

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Browning of milk products during heating and storage is of importance to the dairy industry. A satisfactory method for estimating this color should allow comparisons of the amount present in various types of milk products and of values obtained in different laboratories.

Two types of methods have been used: direct measurement of color by reflectance methods and colorimetric procedures. The former are considered less convenient for general use than the latter because a spectrophotometer is required *(1, 6)*. Choi *et al. (2)* developed a colorimetric method for the determination of color of dry milk products which depends upon tryptic digestion of the milk protein to liberate the color, precipitation of residual protein with trichloroacetic acid, removal of fat and precipitated protein by filtration, and photometric measurement of the color in the filtrate. Patton *(7)* found the method convenient and yielding reproducible results when applied to fluid milk products.

Choi *et al.* recommended the standardization of photometric data with a solution of iodine in 10% potassium iodide, though they recognized that nonadherence to Beer's law of this standard in the wave length region chosen was disadvantageous. The iodine standard proposed by Choi *et al.* has been found to be unstable, and the absorption spectra, in the wave length region used, of the iodine standard and of the colored compounds under study in the milk products are considerably different.

Potassium dichromate was found to have advantages over iodine and is proposed for use as a standard. Generalizing of the procedure and a method of calculation which allows comparison of the amount of color among different types of milk products are desirable. Since browning is associated with chemical changes in the proteins of milk *(3, 4, 5)*, it is proposed that the color of different milk products be compared at the same protein level and that a protein concentration equal to that of cow's milk be employed as the point of reference.

METHODS

An amount of milk or milk product which contains not less than 1.8 or more than 2.2 g. of protein is reconstituted in or diluted with water to make 100 ml. A 25-ml. aliquot of the diluted milk is pipetted into a flask and warmed to 45° C., 1.5 ml. of trypsin solution[1] is added, the flask is stoppered and incubated for 1 hour at 45° C., 1 ml. of 50% trichloroacetic acid and approximately 0.5 g. of Celite No. 505 filter aid (Johns-Manville) are added, and the mixture is filtered by gravity through Whatman No. 2 filter paper, refiltering as necessary to obtain absolute clarity. Absorbance is measured 10 minutes after addition of the tri-

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[1] 1 g. 1:250 trypsin per 50 ml. water.

chloroacetic acid in a colorimeter with a filter transmitting maximally at approximately 440 mμ. The measurement is made against a reagent blank prepared as the milk sample, but with 25 ml. of water replacing the 25-ml. aliquot of diluted milk.

The procedure is standardized as follows:

One hundred mg. of potassium dichromate crystals, primary standard reagent grade, are made to 1 l. with distilled water. The absorbance of this solution is measured in the colorimeter, with distilled water as a blank. The magnitude of one color unit is arbitrarily designated as one-tenth of the absorbance of the potassium dichromate reference solution.

Use of the following method of calculation allows comparison of color of different milk products at equal protein concentrations:

$$\text{Color units of a milk product with a protein concentration equal to that of milk} = \frac{Lu}{0.1\,Ls} \cdot \frac{3.35}{Pu} \cdot 1.1$$

where Lu = absorbance of filtrate examined.
Ls = absorbance of potassium dichromate standard solution.
3.35 = average value for protein content of milk in g/100 ml. This value is equivalent to 3.25% (w/w).
Pu = protein concentration in g/100 ml. of diluted sample.
1.1 = value of dilution ratio $\frac{27.5}{25}$, resulting from dilution of a 25-ml. aliquot of diluted sample with 1.5 ml. of trypsin solution and 1 ml. of trichloroacetic acid solution.

RESULTS AND DISCUSSION

Absorption spectra of filtrates prepared according to the described procedure with sterilized and unsterilized evaporated milk were developed with a Beckman model DU spectrophotometer. Measurements were made in 1.0-cm. matched quartz cells against reagent blanks. Figure 1 shows these curves and one representing the difference in absorbance before and after sterilization. Absorbance in part of the near ultraviolet was so strong that it was necessary to dilute the filtrates for measurement in that region. It is evident that photometric measurement in the near ultraviolet would result in the detection of very slight color changes, not measurable in the visible region. The sensitivity of the method is increased by shifting the region of photometric measurement from 485 mμ, as recommended by Choi *et al. (2)*, toward shorter wave lengths where the compounds formed during browning absorb light more strongly.

Absorbance in the region 390 mμ to 500 mμ by the iodine standard and by the potassium dichromate standard was studied with the spectrophotometer; 1.0-cm. matched cells were used. The absorption spectra are plotted in Figure 2 along with the 390-500 mμ portion of the curves obtained with the sterilized and unsterilized evaporated milk filtrates shown in Figure 1. Also plotted in Figure 2 are transmittance curves of the two glass filters used in the filter photometers employed in this study. Transmittance of the filters against an air path was measured in the spectrophotometer. It is evident that potassium dichromate is

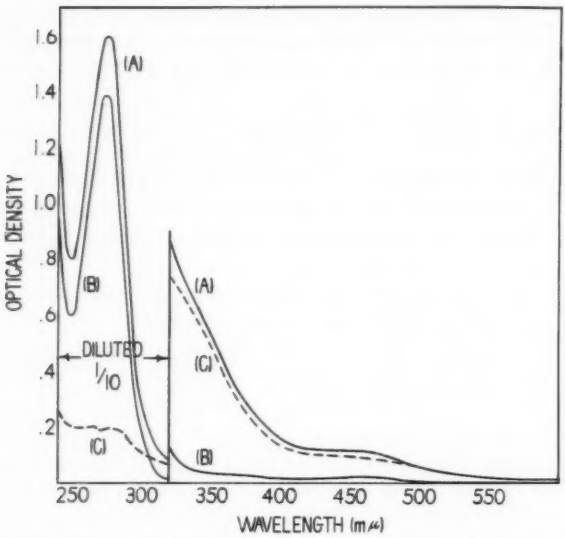

FIG. 1. Change in absorbance during sterilization of evaporated milk: (A) sterilized, (B) unsterilized, (C) change during sterilization (A – B).

the better standard for measurement in filter photometers, since the spectral characteristics of the dichromate solution more closely resemble those of the colored compounds being determined, especially in the spectral region recommended. Photometric measurement with a filter which exhibits maximum transmittance near 440 mμ appears to be the best for use with a potassium dichromate standard. The importance of using a matching standard, to obtain best possible inter-instrument agreement between filter colorimeters, is demonstrated by the difference in spectral transmittance limits and wavelengths of maximum transmittance of the two "440" filters.

The reason for standardization of photometric data is to permit comparison of results obtained in different laboratories or results obtained with different instruments in the same laboratory. To investigate the difference in values obtained with instruments using different though similarly designated filters, colorimetric data were obtained by the procedure described with samples of commercial evaporated milk in two Klett-Summerson photoelectric colorimeters, an Evelyn colorimeter, and the spectrophotometer. Spectrophotometric readings were taken at 440 mμ and colorimetric measurements were made by using the filters described in Figure 2, the same filter being used in both Klett colorimeters. The mean results of closely agreeing duplicate determinations are shown in Table 1. The data of Table 1 demonstrate that the greatest influence on comparisons is exerted by differences in light filters, since the results obtained with the Evelyn colorimeter were considerably different from those obtained with the Klett colorimeters. This result is to be expected, since the filter used in the

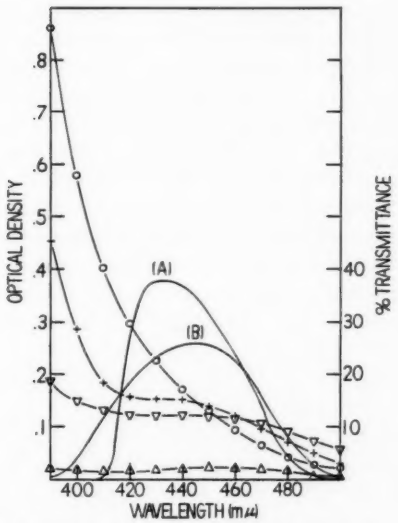

FIG. 2. Similarity of spectral characteristics of potassium dichromate and heat-produced compounds in evaporated milk as compared with iodine standard. ○ = 1 mg. iodine per 50 ml. 10% potassium iodide against 10% potassium iodide, + = 100 mg. potassium dichromate per liter against water, ▽ = sterilized evaporated milk filtrate, △ = unsterilized evaporated milk filtrate. Transmittance of glass filters: (A) = Evelyn No. 440 (Corning No. 3389, 5113-2), (B) = Klett-Summerson No. 44.

Evelyn colorimeter transmitted a narrower spectral band than the filter used in the Klett colorimeters. Since an arbitrary standard must be used, it is apparent that only with the use of spectrophotometers operating on narrow spectral bands and accurately calibrated for wave length or with colorimeters using identical filters will close agreement be possible.

Adherence of potassium dichromate to Beer's law and the linearity of the photometric scales of the instruments used in the investigation were studied.

TABLE 1

Comparison of standardized color results obtained with filter photometers and spectrophotometer

Evaporated milk sample	Klett-Summerson No. 1		Klett-Summerson No. 2		Evelyn photoelectric colorimeter		Beckman model DU spectrophotometer
	(Color) [c]	*(%)* [d]	*(Color)* [c]	*(%)* [d]	*(Color)* [c]	*(%)* [d]	*(Color)* [c]
Brand A [a]	18.0	119.2	18.4	121.9	16.7	110.6	15.1
Brand A [b]	24.6	118.3	24.1	115.9	22.5	108.2	20.8
Brand B [a]	17.0	114.9	17.4	117.6	16.0	108.1	14.8
Brand C [a]	16.0	117.6	16.5	121.3	14.6	107.4	13.6
Average		117.5		119.2		108.6	

[a] Fresh sample.
[b] Aged sample.
[c] Units of color at protein content of 3.35 g/100 ml.
[d] Per cent of spectrophotometric result.

Seven solutions of varying concentrations of potassium dichromate from 25 to 250 mg. per liter were prepared and examined against water in the spectrophotometer at 440 mμ, in one of the Klett colorimeters with the No. 44 filter, and in the Evelyn colorimeter with the No. 440 filter. Absorbance was found to be directly proportional to concentration through the entire range of concentration in the Klett colorimeter and in the spectrophotometer. In the Evelyn colorimeter absorbance deviated from linearity above 160 mg. potassium dichromate per liter.

The stability of the 100 mg. potassium dichromate per liter reference standard was checked by examination in the Evelyn colorimeter immediately after preparation, after 1 month and after 2 months. The *L* values obtained were 0.239, 0.241, and 0.238, respectively. A solution of 1 mg. iodine, Mallinckrodt, analytical reagent, per 50 ml. 10% potassium iodide was examined against 10% potassium iodide in 1.0-cm. cells in the spectrophotometer at 440 mμ immediately after preparation and again after 17 hours. A decrease in absorbance from 0.172 to 0.158 was observed during the 17-hour period.

Celite No. 505 filter aid was used during filtration because it demonstrated maximum ability among several filter aids tested, including Celite analytical filter aid recommended by Choi *et al. (2)*, to produce clear filtrates from unheated or only slightly heated milk samples with a minimum of refiltering. No significant effect on the amount of color present in filtrates was detected when as much as 1 g. of this filter aid was used.

The effect of substrate concentration during tryptic digestion on the accuracy of the method was studied. When up to 4% protein was present in the sample, values obtained agreed closely; above 4% protein, color liberation was incomplete.

TABLE 2

Color of milks and products of milk

Sample	Source	Total color[a]	Heat-produced color[a]
Raw skimmilk		4.9[b]	0
Condensed skimmilk	commercial	6.3	1.4
"Instant" nonfat dry milk solids	brand A	8.3	3.4
"Instant" nonfat dry milk solids	brand B	8.4	3.5
Nonfat dry milk solids	brand C	6.9	2.0
Spray nonfat dry milk solids (low heat)	commercial	5.6	0.7
Spray nonfat dry milk solids (low heat)	commercial	6.3	1.4
Spray nonfat dry milk solids (high heat)	commercial	6.4	1.5
Spray nonfat dry milk solids (baking grade)	commercial	6.1	1.2
Roller nonfat dry milk solids	commercial	9.3	4.4
Roller nonfat dry milk solids	laboratory prepared	7.2	2.3
Raw whole milk		6.5[c]	0
Pasteurized whole milk (144°-146° F. for 30 min.)		6.5	0
Spray whole milk solids	commercial	8.7	2.2
Roller whole milk solids	laboratory prepared	8.6	2.1
Evaporated milk	commercial	19.1[d]	12.6
Dry whey solids (feed grade)	commercial	22.6	

[a] Units of color at protein content of 3.35 g/100 ml.
[b] Average of determinations on eight samples from mixed herd milks, range 4.6-5.1.
[c] Average of determinations on three mixed herd samples.
[d] Average of the results obtained with Klett colorimeter No. 2 shown in Table 1.

The findings of Patton *(7)* regarding the increase in light absorbance by the filtrate with time were confirmed. The amount of trypsin used was reduced from that recommended by Choi *et al.* approximately in proportion to the decrease in solids of the sample digested.

Table 2 lists the color results obtained on samples of nonfat and whole milks and some dry milk products. Values shown are the means of closely agreeing duplicate determinations made with the Klett colorimeter No. 2. Columns show total color and heat-produced color, the latter representing the difference between total color and the average native color of the raw milk. It is evident that sterilization of evaporated whole milk as currently accomplished results in considerably more browning than the drying processes in use.

SUMMARY

A study of a method for the determination of color of milk products has led to the following recommendations for improvement of the method:

1. Use of potassium dichromate instead of iodine in potassium iodide as a standard because of greater stability and because spectral characteristics in the wave length region used for photometric measurement are more like those of the colored compounds being determined.

2. Increasing the sensitivity of the method by shifting the region of photometric measurement toward shorter wave lengths where the compounds being determined absorb light more strongly.

3. Generalizing the procedure and applying a method of calculation which permits comparison of the color of different milk products on the basis of a protein content equal to the average value for protein content of milk.

Data have been presented to show the effect of differences in characteristics of light filters upon the values obtained with different types of colorimeters as compared to spectrophotometric results. Color results obtained on samples of nonfat and whole milks and some dry milk products are shown, and the effect on color of current milk sterilization and drying techniques is indicated.

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THE EFFECT ON FLAVOR OF USING SUBSTITUTE FATS[1] IN DRY WHOLE MILK[2]

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During recent years both the number of uses and the consumption of nonfat dry milk have increased. Principal among the tailor-made products are those for bread baking, cottage cheese making, and home use. No commensurate advancement has been made in dry whole milk. Although there are many factors involved, it seems that flavor has been a critical consideration in the rejection of the fat-containing product by the American housewife. Recognizing that the primary difference between the two milks is the presence of fat, the dairy industry has made every effort to protect the fat of dry whole milk from oxidation during processing and storage. These measures appear to have been inadequate from the standpoint of flavor.

Hetrick and Tracy *(1)* state that a stale flavor develops early in the storage life of gas-packed dry whole milk and that this off-flavor does not resemble the flavor of fat oxidation. This general observation has been made in several other investigations *(3, 4, 9)*. Mussett *et al. (4)* found that the characteristic stale flavor did not develop when milk fat was completely replaced with coconut fat in dry whole milk. With the ready availability of a number of refined domestic fats, it was considered of interest to determine whether other fats would give a type of flavor deterioration in dry milk similar to that of milk fat.

EXPERIMENTAL PROCEDURE

Skimmilk, freshly separated, from mixed herd raw milk at the Pennsylvania State University Creamery was used as a source of solids not fat. Milk for the whole milk powders was secured at the same source and time. Substitute fats used in the investigation were obtained from large domestic processors and were mainly of the type used in vegetable-fat frozen desserts. To characterize these fats the following constants were determined: *(a)* Iodine numbers were ascertained by the Wijs method as described in Triebold *(8)*. Average values of replicates were calculated. *(b)* The capillary tube method described by Triebold was used to determine melting points. Mean values of replicates were calculated.

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[1] Fats other than milk fat were employed in this research as one of a number of approaches to the problem of flavor in dry whole milk. The findings reported do not constitute an endorsement for the use of substitute fats in dairy products either by the authors or the institutions which supported this investigation.

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TABLE 1
Constants of the fats investigated

Type of fat[a]	Iodine No.	Melting point	Refractive index (N_D^{40})
		(° F.)	
Coconut (unhydrogenated)	15	77	1.4505
Coconut (hydrogenated)	4	104	1.4490
Cottonseed No. 1	84	70	1.4635
Cottonseed No. 2	0	145	——
Cottonseed No. 3	80	79-91	1.4605
Cottonseed No. 4	95	86-95	1.4618
Peanut No. 1	85	95	1.4615
Peanut No. 2	0	142	——
Soybean	103	104-113	1.4619
Lard	53	113	1.4589
Butterfat	33	——	1.4550

[a] All fats except refined coconut and butterfat were at least partially hydrogenated. Butterfat was not used as such; the values for it are representative of the fat in the whole milk employed.

(c) Refractive indices were determined with an Abbe refractometer. Readings were taken at 40° C. These data are given in Table 1. Most of the fats initially were emulsified in nonfat milk at a level of 4% and appraised for flavor. Those with definite off-flavors were excluded from the investigation. This simple organoleptic test, which has been used for several years at this laboratory, is a very sensitive means of evaluating flavor in a fat.

The milk was preheated at 150° F. for 20 minutes in a stainless steel, steam-jacketed vat. Condensing was conducted in a Rogers 16-in. stainless steel vacuum pan. The milk was concentrated to approximately 35% total solids at 127° F. pan temperature and under a vacuum of 24 to 25 in. of mercury. The condensed product was heated to 150° F. and homogenized three times at 1,000 p.s.i. single stage, with sufficient fat to give a fat to solids-not-fat ratio of 1 to 2.25. The homogenized product was cooled to about 40° F. on a small surface cooler and then held in a cold room for drying the next day. The drying operation was accomplished in a Rogers box-type drier equipped with a single No. 72 atomizing nozzle. During the operation, air inlet and exhaust temperatures were maintained at approximately 295° and 190° F., respectively. A Cherry-Burrell viscolizer was used as a positive pump (600 p.s.i. pressure) to atomize the product at 150° F. into the drier. The product was immediately removed from the drier and stored for a short time at –5° F. until scored for flavor and packed. The finished product was sifted through a conventional flour sifter. No. 1 cans, holding about 180 g. of dry milk, were employed for air-packed samples. No. 2 cans, holding about 250 g. of powder, were used for vacuum-packed samples. The samples to be vacuum packed were placed in an anaeroid can and evacuated to a level of 25 in. of mercury. The milk powder was allowed to equilibrate for 24 hours. This process was repeated. At the end of the 48-hour period the samples were vacuum sealed, at about 23 in. mercury, with an American Can Company machine. All samples were stored at room temperature.

The nine-point hedonic scale developed by the Acceptance Division of the

Quartermaster Food and Container Institute *(7)* was used as a basis for scoring the reconstituted dry milks. This scale is described in Footnote 1, Table 2. The dry product was reconstituted in distilled water with the aid of a Waring blendor to yield a 13% total solids beverage. Samples were presented by the aspersion technique described by Patton and Josephson *(5)*. Samples were maintained at 20° C. in a water bath, and a random order of presentation was used. Five experienced observers made two evaluations of each sample. Thus, the average score for a given product is the average of ten evaluations. The observers were also requested to give qualitative flavor descriptions after scoring the samples. After samples dropped to a flavor score of 3.5 or less, no further observations were made. This was done to help minimize the "carry-over" effect from badly deteriorated samples. During most of the evaluations homogenized, fresh whole milk was included to serve as a reference standard for the tasters.

RESULTS AND DISCUSSION

The flavor scores of the dry whole milks and dry milks containing the various substitute fats are presented in Table 2. Where more than one trial was performed with a particular fat, an effort has been made to give representative data. Ideally, all samples should have been prepared from the same milk at the same time. This was physically impossible. All air-packed samples were prepared from early trials during May and June, 1953, and all vacuum-packed samples were prepared in later runs during July to September, 1953. Thus, seasonal variations in the milk supply may be a factor in these data.

Several general observations may be made regarding the data in Table 2. With the exception of dry whole milk, the vacuum-packed samples exhibited better flavor stability than the air-packed samples during storage. In general the vegetable fats, excluding coconut fat, yielded products with poor flavor,

TABLE 2

Flavor scores[a] of dry milks made with substitute fats

Type of fat[b]	Flavor scores after storage[c] (months)									
	Air packed					Vacuum packed				
	Fresh	1	2	6	No. of Trials	Fresh	1	2	6	No. of Trials
Coconut (unhydrogenated)	5.2	5.6	3.1	2.8	1	6.5	5.2	5.8	5.7	6
Coconut (hydrogenated)	5.3	5.2	3.6	3.0	5	6.6	5.9	5.9	5.7	7
Cottonseed No. 1	3.5	3.5	2.2	—	1	3.2	4.5	3.4	2.7	1
Cottonseed No. 2	3.2	1.5	—	—	1	—	—	—	—	0
Cottonseed No. 3	5.1	2.5	—	—	1	—	—	—	—	0
Cottonseed No. 4	5.4	3.7	3.0	—	1	4.9	3.6	3.5	3.3	1
Peanut No. 1	—	—	—	—	0	4.2	6.0	4.6	2.5	1
Peanut No. 2	3.8	3.0	—	—	1	—	—	—	—	0
Soybean	4.8	2.5	—	—	1	4.5	4.1	3.4	2.7	1
Lard	5.0	2.7	—	—	1	5.2	5.2	3.9	3.9	2
Dry whole milk	5.9	4.3	3.0	—	2	5.5	3.5	—	—	7

[a] Flavor evaluation scale: 9—like extremely, 8—like very much, 7—like moderately, 6—like slightly, 5—neither like nor dislike, 4—dislike slightly, 3—dislike moderately, 2—dislike very much, 1—dislike extremely. The evaluation of 44 fresh homogenized milks yielded a score of 6.6.

[b] For certain characteristics of these fats, see Table 1.

[c] Room temperature.

either initially or after a short storage period. In criticizing these products the taste panel used such terms as "beany," "musty," "stale," "oxidized," and "unclean." In contrast to these findings are those for coconut fat, both hydrogenated and unhydrogenated. The impressive performance of these two fats is shown by data for the vacuum-packed samples. Statistical analysis of flavor scores from vacuum-packed samples containing the two coconut fats indicated no significant difference between them. In vacuum-packed trials, peanut oil No. 2 and cottonseed oil No. 2 were excluded. These two products, because of their high melting points, could not be processed satisfactorily. The air-packed products made with them were extremely chalky-tasting.

Dry whole milk showed certain qualities which differentiated it from the dry milks containing substitute fats. The same general flavor criticisms were used by the taste panel for air-packed and vacuum-packed dry whole milks. These included such terms as "heated," "like coconut," "buttery," and "perfumey." These criticisms were used only with reference to dry whole milk. In fact, taste panel members almost infallibly could pick the dry whole milk within a group of samples stored 1 month or more, which demonstrates the uniqueness of flavor deterioration in this product. Development of the off-flavor produced a substantial drop in flavor score during the first month of storage, as shown in Table 3. Some evidence concerning the nature and origin of this off-flavor in milk fat has been published *(2, 6)*.

TABLE 3

Flavor scores and preheat treatment of vacuum-packed dry whole milks

Trial No.	1	2	3	4	5	6	7
Fresh	4.6	4.6	5.5	6.3	5.6	5.5	3.5
Stored 1 mo.	2.2	3.5	3.5	2.4	3.3	2.6	3.0
Preheat[a]	150° F.	150° F.	165° F.	165° F.	165° F.	180° F.	180° F.

[a] All for 20 minutes holding time.

Flavor deterioration in dry milk during the early months of storage has been emphasized in this study. There are many reports in the literature of dry whole milks which show good flavor qualities after 1 to 2 years storage at room temperature. It is possible that these products have been rated in terms of a hypothetical dry milk standard. It seems questionable that such milks could be considered indistinguishable from fresh fluid milk. Forty-four samples of fresh homogenized milk, which were used as a reference standard in this investigation, yielded an average flavor score of 6.6. The only dry milks which approached this value after 6 months storage were those containing coconut fat.

Most of the substitute fats employed in this study were developed primarily for use in frozen desserts. The cold processing and storage treatment of these desserts makes certain characteristics of the fat desirable. These characteristics do not appear suitable for an ideal fat in dry milk. The latter should have a low iodine value and a melting range near body temperature. With the exception of coconut fat, no natural or processed fat having such qualities could be located.

SUMMARY

Flavor deterioration in dry milks made with substitute fats was evaluated and compared with that of dry whole milk. Dried milks were prepared with cottonseed, peanut, soybean and coconut oils, and lard. All of these fats were at least partially hydrogenated with the exception of coconut oil, which was used in both hydrogenated and unhydrogenated forms.

The findings of others *(1, 3, 4, 9)* that vacuum- or gas-packed dry whole milk develops a stale flavor, different from oxidized flavor, during early storage were confirmed. This off-flavor produced a substantial drop in the flavor score of both air- and vacuum-packed dry whole milks during the first month of storage. Such a flavor was not detected in any dry milks containing substitute fats. With the notable exception of coconut fat the substitute fats made dry milks with poor (oxidized) flavor qualities. Vacuum-packed dry milks made with coconut fat, either hydrogenated or unhydrogenated, exhibited good flavor after 6 months storage at room temperature.

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A DEVICE FOR COLLECTING AND RAPIDLY COOLING SAMPLES FROM HIGH-TEMPERATURE SHORT-TIME HEATING UNITS

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The advent of high-temperature short-time (HTST) heating units for the pasteurization or sterilization of milk or ice cream mix has presented the problem of obtaining a satisfactory sample immediately after heating, because of the completely closed design of these systems. The problem is further complicated by the lack of an adequate method of rapidly cooling the sample to prevent the occurrence of bacterial destruction during the cooling period. In formulating a suitable technique for sampling HTST units, these two points must be considered. In the course of studies on the HTST pasteurization of ice cream mix with a Roswell heater, a simple device and technique for collecting and rapidly cooling the samples were developed.

DESCRIPTION OF APPARATUS

The sampling device used in these studies consisted of two parts. The first part comprised the sampling port per se.[1] It consisted of a small stainless steel bushing fitted with a rigidly supported slit, gum rubber diaphragm. The bushing was threaded to permit it to be screwed into a tapped and threaded stainless steel end plate; a rubber washer prevented leakage at this junction. The sampling port was installed in a T-joint and secured by a standard hexagonal capping nut. The T-joint was placed in the line in such a manner that the direct flow of product was against the sampling vent. After each use, the sampling port was removed for manual cleaning. The component parts of the sampling port are shown in Figure 1, items *A* through *D*.

The second part of the sampling apparatus consisted of a product receptacle and a cooling chamber. The product was collected in a 250-ml. Erlenmeyer flask fitted with a No. 6.5 rubber stopper containing a small air vent and 14-gauge, 6-in. hypodermic needle permanently inserted at an angle of about 60 degrees. A 14-gauge needle is desirable with ice cream mix; smaller bore needles may be used with less viscous products. The needle was inserted completely through the stopper so that the shank touched the bottom of the stopper; in this position, the open end of the needle rested against the side of the flask in the neck region and directed the flow of hot product onto the inner surface of the container to facilitate rapid cooling. The assembled receiver was sterilized by autoclaving. To

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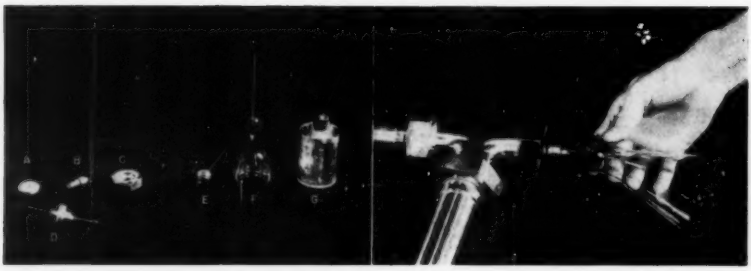
[1] The sampling port was obtained from Sidney P. Foster, Floral Park, New York.



1 2

FIG. 1. Sampling port and flask showing position of needle. A—tapped end plate, B—bushing and diaphragm, C—capping nut, D—assembled port and needle, E—rubber stopper and needle, F—assembled sampling flask, G—complete refrigerated unit with closure omitted.

FIG. 2. Sampling position showing flask with refrigerated container removed to indicate position of needle in the line and flask.

maintain sterility, the exposed shaft of the needle was covered with paper and the air vent was plugged with cotton.

To obtain rapid cooling, the sample vial was placed in a completely frozen water bath or brine. This was prepared by placing the sterilized assembled Erlenmeyer flask in an ordinary No. 6 tin can completely filled with refrigerant solution. A 2-in. piece of a ¼-in. glass rod was placed on the bottom of the can to allow a layer of refrigerant to collect under the flask. A 4-sq. in. section of thin gum rubber sheet was stretched over the neck of the flask and top of the tin container to prevent leakage when the unit was tipped in the act of sampling. A small hole about ¼ in. in diameter was cut in the center of the cover to prevent splitting and obtain a tight seal around the neck of the flask. Rubber bands were used to maintain the flasks in a completely submerged position and to secure the diaphragm to the container.

The entire assembly was cooled by placing the unit in a cold room, maintained at –10° F. overnight. The refrigerants consisted of ice, 23% sodium chloride, or 29% calcium chloride solution. Occasional breakage of the Erlenmeyer flasks was experienced when ice was used as coolant. No breakage occurred with the 29% calcium chloride solution, as it did not solidify. The sampling assembly is shown in Figure 1, items *E* through *G*.

The sample was taken by inserting the needle completely through the diaphragm into the flowing product. The unit was held so that the product collected in the lowest portion of the flask opposite the side at which the needle was located. This is illustrated in Figure 2. By inserting the needle completely through the sampling port a sample was obtained beyond the dead-end of the T-joint. Immediately after the sample was collected, the needle was removed from the line and the container was shaken to facilitate cooling. The duration of the sampling period was standardized to insure uniform volume of sample.

A study was made to determine the rate of cooling of the sample with various sampling times and cooling agents. A copper-constantan thermocouple mounted

on a wooden applicator stick was placed inside the flask approximately 1/8 in. from the bottom in the portion of the flask in which the sample collected. The potential was read with a Rubicon potentiometer. The highest temperature reached during the time the sample was collected and the time required to reach that temperature were recorded. In addition, the temperature of the product was determined 15 seconds after the start of the sampling process to obtain information on the rapidity of cooling.

RESULTS AND DISCUSSION

Table 1 shows the maximum temperatures obtained in sampling when water and ice cream mix were used with three different coolants.

The data indicate that calcium chloride is better than sodium chloride brine as coolant; the latter is better than water. Covering the container when calcium chloride was used undoubtedly aided in cooling. The data also show that the smaller the volume of sample, the better the cooling.

An evaluation of the cooling process can be made by estimating the lethality contributed by it. To undertake this, an organism of known heat resistance should be selected for evaluation. Preferably, it should be one which will have some survivors after treatment at the highest processing temperature. For example, in a study involving the determination of pasteurizing efficiency of milk at high temperatures *Micrococcus freudenreichii* MS66 *(2)* may be used. In similar studies with ice cream mix, *Micrococcus* sp. MS 102 *(1)* would be satisfactory.

TABLE 1

The effect of various refrigerants on the rate of cooling of water or ice cream mix processed in the Roswell heater at a rate of 6,000 lb. per hour

Product	No. of deter.	Sampling period	Vol. of sample	Process temp.	Coolant[a]	Highest temperature	
						During sampling[b]	After 15 sec.[c]
		(sec.)	*(ml.)*	*(° F.)*		*(° F.)*	*(° F.)*
Water	6	6	95-105	200	Ice	141-166 (155)	137-154 (143)
Mix	5	6	85-125	190	Ice	162-170 (166)	137-150 (142)
Mix	3	2	25-30	187	Ice	133-170 (153)	90-108 (99)
Mix	2	4	44-45	187	Ice	154-169 (161)	133
Mix	3	2	18-25	205	NaCl	137-154 (146)	90-99 (94)
Mix	2	4	46-52	205	NaCl	152-165 (155)	129-137 (133)
Mix	5	4	46-50	205	$CaCl_2$[d]	146-154 (148)	104-112 (110)

[a] 23% NaCl and 29% $CaCl_2$ solutions; all solutions were chilled overnight at a temperature of −10° F. before using.

[b] The highest temperature was always obtained at the end of the sampling period; the average temperature is given in parentheses.

[c] This interval was calculated from the start of the sampling period.

[d] Container was sealed with a rubber diaphragm.

With the latter organism the lethality contributed by the cooling temperatures given in Table 1 was determined in the following manner: The lethal rate was considered as the reciprocal of the time required to produce 99.9% destruction of the organism at any given temperature; the total lethality was the summation of the products of lethal rate and time of exposure at all temperatures involved in the cooling process, as shown for a specific case in Figure 3A.

To determine the lethal effect of the cooling process in terms of the percentage destruction of organisms, a theoretical survivor curve was constructed from the z value plot (log time vs. temperature at 99.9% destruction). The two points used to construct this curve were zero time at the 100% survivor level and the time read from the z value plot corresponding to the temperature under consideration. A different survivor curve was drawn for each temperature to which the sample was cooled. From each curve the percentage of destruction during the cooling process may be read directly from the plot. This is illustrated by a specific example in Figure 3B.

The fraction of the surviving organisms may be calculated by means of the following equation:

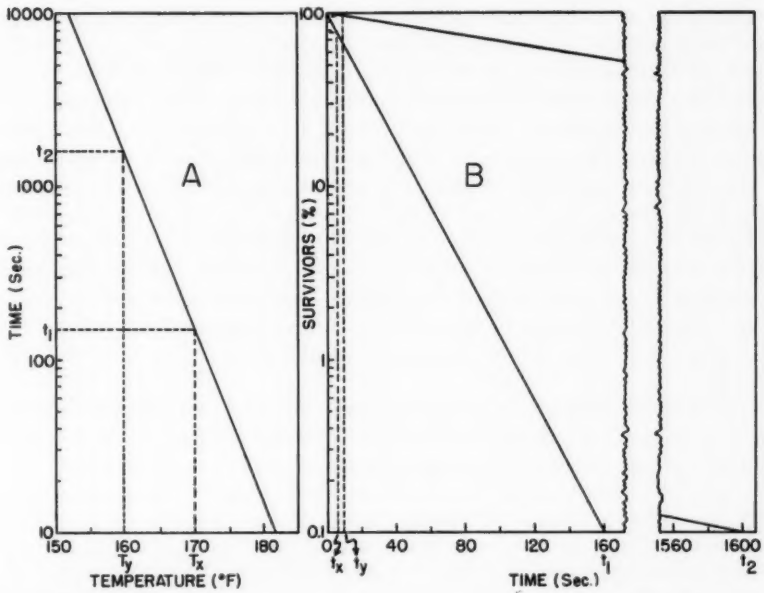
$$\text{Log of fraction surviving} = -3 \times \text{lethality}$$

where –3 is the log of the fraction of surviving organisms on which the z plot is based. The plot in Figure 3A was based on a fraction of 0.001 organisms surviving. Values obtained by calculation are in good agreement with those picked from the graph in Figure 3B.

To determine the maximum lethal effect and the percentage of destruction contributed by the cooling process, the most rigorous conditions were selected. Although the highest temperature was not reached until the end of the sampling period, this was assumed to prevail throughout the entire sampling period, and this temperature was used in calculating the lethality value.

As shown in Table 1 and Figure 3A and 3B, when ice cream mix was processed at 190° F. and sampled for a 6-second period with ice water as coolant, the highest temperature reached in any trial was 170° F.; 9 seconds later the maximum temperature was 150° F. The lethality contributed in this sampling process under the most rigorous conditions prevailing throughout the 6-second period was 0.0375. In calculating the lethality contributed during the 9-second cooling period, it was assumed that an average temperature of 160° F. prevailed. This contributed a lethality of 0.0056. The total lethality contributed in the entire sampling process was 0.0431. This is equivalent to a 26% destruction of the organisms present at the beginning of the cooling period.

When the process temperature of ice cream mix was 205° F. and a 4-second sampling period was used with calcium chloride solution as refrigerant, the maximum temperature obtained in any trial was 154° F. The lethality contributed under these conditions was 0.0006. This is equivalent to approximately 1% destruction of the number of organisms present at the start of the cooling period. No lethality was contributed at a temperature of 112° F.



Product temperature		190° F.
Temperature for first 6 seconds (t_x) of cooling		170° F. (T_x)
Temperature after 15 seconds (t_y) of cooling		150° F. (T_y)
Average temperature for 9-second cooling period	$(T_x + T_y)/2$	160° F. ($T_{av.}$)

Total lethality of cooling process (from Plot A)

$$\text{Total lethality} = \left(\frac{1}{t_1} \times t_x\right) + \left(\frac{1}{t_2} \times t_y\right)$$

Lethality at $t_x = 0.0375$
Lethality at $t_y = 0.0056$
Total lethality = 0.0431

Per cent destruction during cooling process (from Plot B)

t_1 and t_2 are obtained from Figure 3A.
% survivors at $t_x = 77$
% survivors at $t_y = 97$ of surv. at t_x
% total destruction = 26

Per cent destruction obtained by calculation

Log of fraction surviving = –3 times lethality = $-3 \times 0.0431 = -0.1293$
Log^{-1} of fraction surviving = 0.7425
% destruction = 25.75

FIG. 3. Graphs showing calculations of lethality and per cent survivors of *Micrococcus* sp. MS 102 with the data from one trial in Table 1.

To determine the adequacy of a cooling process, the total destruction obtained during the heating should be taken into account. Thus, when the initial level of organisms is two million per milliliter and 5%, or 100,000, survive the heat processing treatment, a cooling procedure which destroys 26% of the survivors would indicate a destruction of 96.3% instead of 95%. On the other hand, a cooling procedure which is responsible for the destruction of only 1 or 2% of the organisms present at the beginning of the cooling period would cause only negligible destruction in terms of the original number of organisms.

The sampling method may be modified by collecting the sample directly into a sterile flask containing a chilled dilution liquid. The amount of product collected would have to be ascertained by weighing the filled flask and from this value subtracting the weight of the flask and dilution liquid. This technique was not investigated in the present study, but it would be expected to produce satisfactory results in tests in which dilution of the samples would cause no complications.

The choice of a cooling process is determined by the differences in bacterial counts that may be detected by the plating methods used, and its adequacy may be determined by the procedure outlined above. It may not be necessary in all cases to seek the best possible cooling procedure because bacterial plate counts are usually relied upon to show only differences of the order of 25%. The procedure is also applicable in determining the adequacy of the cooling rate in studies of other heat-induced phenomena which may require rapid and efficient cooling.

The sampling device described here would reduce the error in bacterial counts due to slow cooling, normally associated with the sampling of milk and milk products at high temperatures under plant conditions.

SUMMARY

A device is described for collecting and cooling samples processed in HTST units for bacteriological examination. A method is given by which the adequacy of a cooling process may be evaluated.

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THE DEVELOPMENT OF RUMEN FUNCTION IN THE DAIRY CALF

I. SOME CHARACTERISTICS OF THE RUMEN CONTENTS OF CATTLE OF VARIOUS AGES[1,2]

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Little work has been done to estimate the age at which calves under normal management develop a rumen function that approximates that of the adult. Kesler *et al. (3)* found that by 6 weeks the color, consistency, and odor of the rumen contents were similar to these characteristics in the adult. McCandless and Dye *(5)* estimated from blood sugar values that rumen function became important around 6 weeks of age. Pounden and Hibbs *(9)* found that they could cause the establishment of adult-like rumen organisms as early as the third week of life by the use of cud inoculation and diets high in hay.

The object of this work was to characterize rumen function in the adult and to determine characteristic differences between animals of different ages. The lack of clear-cut, specific criteria of adult rumen function presents a problem in a study of this type. However, it was believed that a reasonably satisfactory indication of adult rumen function might be obtained from the following criteria:

1. Some measure of the ability to digest cellulose
2. A description of the more characteristic types of bacteria
3. An estimate of the total bacterial population
4. Numbers of protozoa
5. A measurement of some of the rumen acids

METHODS

All samples were collected by stomach tube about 6 hours after feeding. The color and odor of the sample were noted. The sample was strained through a single layer of cheesecloth and squeezed to about 24% dry matter. One g. of this solid was added to 99 ml. of sterile tap water. This solution was flushed with nitrogen and shaken for 3 minutes in a mechanical shaker. This was considered a 10^{-2} dilution. Further dilutions were then made up to 10^{-6} in sterile tap water. The 10^{-2} dilution was used for making slides for identification of characteristic types of bacteria, the 10^{-4} dilution for making slides for total bacterial counts, and the 10^{-4}, 10^{-5}, and 10^{-6} dilutions for aerobic plate counts. The liquid portion

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of the sample was used for protozoa counts and for cellulose digestion and rumen acid determinations.

For identification of the characteristic types of bacteria, the Pounden and Hibbs classification as modified by Rusoff *et al. (10)* was used. The classification was further modified by adding another group of G^- spiral-like organisms about 4 to 18μ in length and 0.5μ or less in width. This was called the "spiral" group. For microscopic counts three 1-cm. square areas were marked off on slides. Over this area, 0.01 ml. (standard loop) of the 10^{-4} dilution was spread evenly, dried, and fixed. It was then stained with 2.5% Safranin solution. For counting, the oil immersion objective (1.8 mm.) was used in combination with a 10× ocular. At least 30 fields on 2 squares were counted. For aerobes, counts were made by the standard plating procedure for milk on Difco tryptose glucose extract agar media. Triplicates of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were prepared.

The problem of determining cellulose digesting ability was a difficult one. Two methods were used. One was similar to the cellulolytic method developed by O'Neil *et al. (8)*. A ½-in. piece of 4-mm. glass tubing was used as a weight. For cellulose a two-strand length of white darning cotton was tied to the weight, which was then suspended in about 10 ml. of rumen fluid in a 16-mm. test tube. The surface was then covered with paraffin oil and the tube was closed with a rubber stopper. The tubes, prepared in duplicate, were incubated at 39° C. and examined daily until the strings were broken.

The other method was similar to that of McAnally *(4)* and made use of gas production as a measure of the ability of the rumen fluid to ferment carbohydrates. In this method 0.2 g. of a vegetable cellulose (Solka Floc) in a 50-ml. Erlenmeyer flask was mixed with 10 ml. of rumen fluid and 0.02 g. of cysteine. To measure the gas produced, an inverted, calibrated 50-ml. centrifuge tube, filled with water, was connected atop the Erlenmeyer flask by means of a system of rubber stoppers. Glass tubing conducted the gas produced up into the water-filled centrifuge tube, displacing water through an outlet near the bottom. Before collecting gas, the flask was flushed with nitrogen. Two such setups containing Solka Floc and two without any added cellulose constituted a test. A 24-hour incubation at 39° C. was used at all times.

For the protozoa count, the rumen fluid, after being filtered through cheesecloth, was diluted with an equal volume of 0.01% solution of gentian violet containing 2% glacial acetic acid. After thorough mixing, the solution was allowed to flow under the cover slip of a Spencer Brightline hemocytometer and the protozoa were counted in duplicate.

For analysis of rumen acids the chromatographic method of Neish *(7)* with the modification of Bauman *(1)* was used, dispensing with ether extraction in accordance with the findings of Harper *(2)* with cheese and Wise *(12)* with aqueous solutions of fatty acids. This modification consisted of pipetting 1 ml. of the acidified (1.5 pH), clarified sample onto 1 g. of dry silicic acid, which had been placed on top of the column. The acids determined were acetic, butyric, propionic, formic, and a succinic-lactic fraction. Succinic and lactic acid fractions were determined together as one group because of incomplete separation.

DESIGN OF EXPERIMENT

For this experiment six age divisions of animals were made as follows: 0-1 month; 1-2 months; 2-3 months; 5-6 months; 10-12 months; and lactating cows over 2 years. At least five animals were sampled for each group. The animals were all kept under the routine conditions of feeding and management of the University of Wisconsin herd.

The calves received up to 6 lb. of whole milk fed twice daily from open pails. Hay and a 16% protein concentrate, mixed with a pelleted high protein calf starter, were kept before the calves, with a limit of 3 lb. of concentrate per day per calf. When the calves were 6 months of age, milk was removed from the ration, and the feeding of corn silage was instituted. The concentrate was then limited to 4 lb. per day of 16% protein feed per animal, which was maintained until the animals were milking. Hay and water were available at all times.

The adult animals received from 8 to 16 lb. of 16% protein concentrate mix, from 30 to 40 lb. of corn silage, and about 15 lb. of mixed hay per day.

RESULTS

All of the samples obtained contained solids in amounts sufficient for analysis, which indicated that even the youngest calves were eating some hay and grain. The samples from the adult, 12-, 6-, and 3-month-old groups were uniform as regards color, odor, and hay content. The samples from the 2- and 1-month groups varied greatly in physical characteristics. Most of them had an offensive odor and were composed largely of grain and coagulated milk, with some hay.

The results are shown in Table 1. The two methods used for measuring cellulolytic activity of the rumen fluid have been described. The rumen fluid from all of the four adult animals sampled digested the cotton string to the breaking point within 3 days, whereas that from the younger animals, especially the 1-month-old group, required a longer time. A graded response in gas production from Solka Floc was noted between the different age groups, with the older groups producing the greatest volume. By means of the analysis of variance *(11)*, it was found that by either measure, at the 5% level of probability, the 1-, 2-, and 3-month-old groups were significantly different from the adult group.

The average protozoa count showed a similar graded response with a high in the 1-year-old group. Statistically, the 1- and the 2-month groups were different (5% level) from the adult group.

There was no significant difference among the groups in aerobic count. The microscopic count showed, for the most part, an increase in numbers with age. Statistically, the 2- and 3-month groups were different at the 5% level from the adult group. No other differences were apparent.

Some striking differences were detected among the types of bacteria present — as characterized by the Pounden and Hibbs classification. The adult, 12-, and 6-month groups showed indicator organisms that were characteristic of a hay type of diet, whereas the rest of the groups showed a grain type of flora. The 1- and 2-month groups showed a scarcity of both Hay I and Hay II groups. Only

TABLE 1

Characteristics of the rumen contents by age groups of animals on normal diet

Age group	No. in group	Rumen acids—m*M* per l. (Mean value and standard deviation of the means)					
		Butyric	Propionic	Acetic	Formic	Succinic-lactic	Total
Adult	7	14.49 ±2.67	18.08 ±2.60	63.01 ±14.18	1.29 ±0.18	5.24 ±1.55	102.01 ±18.16
12 mo.	6	18.01 ±2.88	16.29 ±4.23	67.74 ±11.52	1.24 ±0.29	6.78 ±2.85	110.04 ±15.99
6 mo.	5	22.39 ±4.03	18.73 ±2.56	73.26 ±7.84	2.53 ±1.85	13.46 ±4.58	130.39 ±6.90
3 mo.	5	17.87 ±4.03	26.83 ±12.73	75.01 ±7.96	1.46 ±0.26	6.13 ±1.66	127.32 ±15.36
2 mo.	5	18.60 ±4.27	32.41 ±11.17	69.72 ±10.14	1.49 ±0.35	6.55 ±1.57	128.78 ±10.66
1 mo.	5	16.24 ±8.24	25.19 ±20.13	48.80 ±12.93	5.47 ±5.92	55.96 ±60.80	151.65 ±41.47

Age group	No. in group	Digestion of string (days) [a]			Gas from Solka Floc	Protozoa	Aerobes	Microscopic count	Pounden and Hibbs Classification [b]							
		<3	<6	<15					Hay I	Hay II-a	Hay II-b	Hay II-c	Grain I	Grain II	Misc. I	Spiral
					(ml.)	*(thousands per ml.)*	*(millions per g.)*	*(billions per g.)*								
Adult	7	4	0	0	9.4 ±4.4	477 ±121	9.9 ±7.6	66.1 ±19.1	7	2	0	4	4	6	6	7
12 mo.	6	–	–	–	7.3 ±2.8	843 ±289	7.4 ±4.0	53.3 ±14.6	5	0	0	5	3	6	6	6
6 mo.	5	1	2	2	5.4 ±4.5	738 ±160	15.2 ±13.7	44.5 ±14.2	5	0	0	5	2	5	5	5
3 mo.	5	1	1	2	3.8 ±4.9	659 ±553	3.6 ±3.1	37.8 ±15.7	1	0	0	3	3	5	5	4
2 mo.	5	2	1	2	4.2 ±4.7	137 ±139	7.1 ±7.2	28.5 ±16.8	1	0	0	1	3	5	4	5
1 mo.	5	0	1	0	0.8 ±3.4	0 ±0	12.9 ±18.0	46.3 ±19.0	1	0	0	1	3	4	1	2

[a] For digestion time of cotton string, the adult group contained 4 animals, the 12 mo. group none, all others 5 each. Figures indicate number of animals within each time period.

[b] Each figure indicates the number of animals in the group having the organism present.

one calf of the five in the 3-month group yielded the Hay I organisms, but three of the five carried the Hay II type. The level of the Grain I and Grain II groups was about the same for all calves. The Miscellaneous I group of Rusoff was absent in four of the five calves, as were the spiral organisms in three of five calves in the 1-month-old group.

There were no statistically significant differences between the groups in the

absolute amounts of propionic and butyric acid. The total amount of acetic acid was significantly lower (5% level) in the 1-month-old group than in the others. For formic acid, the 1-month-old group was significantly greater (5% level) than that of all other groups, except the 6-month. Also, the 1-month group showed significantly greater amounts of succinic and lactic acids than the others. The 1-month-old group had significantly greater (5% level) amounts of combined acids than either the adult or 12-month groups. When the data of the rumen acids were converted into percentages of the total acids titrated, a similar picture was presented.

DISCUSSION

The data, though showing large variations within age groups, indicate that the development of an adult-like rumen function is gradual and starts early in the life of the calf. These changes can best be attributed to changes in the diet. The newborn calf appears to be without any of the characteristic bacteria of the adult. Many different types of bacteria are ingested by the calf, but not all of these grow in the rumen. The growth and predominance of an organism seem to depend to a large extent upon the substrate present *(9)*. As this substrate changes with changes in the diet, so does the type of organism that predominates.

Milk constitutes the major part of the diet of the very young calf, and the 1-month group shows what happens at this time. The composition of the flora varied, and many organisms characteristic of the adult bovine were not found. There was little digestion of cellulose, presumably because of absence of the cellulolytic organisms. Protozoa were absent, either because of a lack of infection, or because the milk diet was unfavorable for their growth. The aerobic count of the calves did not differ greatly from the adult count. The rumen acids of the calves, perhaps reflecting the composition of the flora and the lack of cellulose in the diet, differed greatly from the rumen acids in the adult. Noticeably, the acetic acid was lower, whereas large amounts of formic acid and of the succinic-lactic group were present.

The 2- and 3-month groups clearly showed a transition toward the adult function. Cellulose digestion, though low, increased decidedly from that of the 1-month group. A protozoan infection occurred and a tremendous increase in numbers took place. Aerobes had decreased, as had the total numbers of bacteria. Bacteria representing the hay type of organisms were present only to a limited extent, but decidedly on the increase. The rumen acids could not be statistically distinguished from the adult values.

For these animals, hay and grain were making up an increasing part of the diet. However, the grain feeding was very liberal, but hay consumption was not emphasized. This high grain to hay ratio may have been responsible for the failure of the Hay I organism to appear and for the rather slow rate of cellulose digestion as measured in vitro. The protozoan numbers were high, which is in accordance with the observation of Mowry and Becker *(6)* that the protozoa tend to live up to the limit of their environment.

The data from the 6-month, 12-month, and adult groups indicate a progressive trend, for most factors determined, toward the adult values. The 6-month group probably reflected the fact that milk was still included in the diet and that the feeding of silage, in small amounts, had started. Except for the protozoa, the 12-month and adult groups were very similar.

SUMMARY

The data presented show a picture of gradual but progressive development of rumen function with age in dairy animals. Large within-group variations tended to make apparent differences from one age group to the next nonsignificant statistically. However, the amount and percentages of the acids investigated in the rumen contents of these calves on a normal diet tended to stabilize at least by the second month of life. According to this and the other criteria used in this work, by about 6 months of age few differences existed between the calf and the adult under this feeding regime.

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CAROTENE IN THE RATION OF DAIRY CATTLE. I. THE INFLUENCE OF LONG PERIODS OF SUBOPTIMAL CAROTENE INTAKE ON THE CAROTENE AND VITAMIN A VALUES OF THE BLOOD, LIVER, AND MILK FAT OF DAIRY COWS[1,2]

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Blood carotene and vitamin A values have been used for some time as a measure of the carotene and vitamin A nutritional status of livestock. Of late, some doubts have been raised as to the relationship between the blood values and the true nutritional level with respect to carotene and vitamin A. Recent investigations have suggested that liver vitamin A concentration is a better indicator of vitamin A and carotene intake than is blood plasma vitamin A *(7, 13)*. Ronning *et al. (11)* found wide variations in blood carotene levels. Five animals with an average daily carotene intake of 70 γ per pound of body weight maintained blood plasma carotene values ranging from 200 to 945 γ per 100 ml.

Although the liver has long been recognized as the main storage organ of vitamin A in the body, the fact has been of little use to the investigator. Until the development of the liver biopsy technique by Seghetti and Marsh *(12)*, Whitehair *et al. (15)*, and others, liver samples were available for analysis only at slaughter or post-mortem. Baker and associates *(1)*, using liver biopsy technique with beef cows, found that 330 γ of carotene per pound fed during lactation gave lower vitamin A liver values than 60 γ of carotene per pound fed during gestation without lactation. This indicates that the stress of lactation depletes liver stores of vitamin A to a greater extent than does gestation.

Hodgson *et al. (9)* found on slaughter of two bulls fed low carotene hay containing 1.7 to 1.8 p.p.m. of carotene that the livers contained 4 and 30 γ vitamin A and 5 and 50 γ carotene per gram of liver. Bratton *et al. (3)* reported the livers of three bulls fed carotene-deficient rations to have 5 to 13 γ of vitamin A and 30 to 50 γ of carotene per gram of fresh liver.

Comparatively little is known of the metabolism and utilization of carotene and/or vitamin A in dairy cattle. In connection with a study *(4, 5, 6)* of carotene in the ration of dairy cattle at the Oregon Agricultural Experiment Station it was found that certain animals which had a suboptimal intake of carotene for a number of years did not respond to added amounts of carotene in the diet.

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[2] Regional Cooperative Research W-2, Causes and Prevention of Breeding Failure, California, Colorado, Idaho, Washington and Dairy Research Branch USDA Cooperating.

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This paper reports a study of the metabolism of carotene and vitamin A in the ration of dairy cattle by means of analyses of blood, liver, and the milk fat of control cows and cows that have been subjected to suboptimal carotene intakes while in utero and subsequently through two or three lactations.

EXPERIMENTAL PROCEDURE

In this study eight cows were used, of which five were from dams receiving normal rations. The dams of the three other cows (251, 267, and 553) received 50 γ carotene per kilogram body weight during the last 4.0, 7.9, and 5.0 months, respectively, of their pregnancies.

Blood samples were taken at regular intervals and analyzed for carotene and vitamin A by a modification (mild saponification) of Kimble's method *(10)*. Liver samples were taken by the biopsy technique as developed by Bone *(2)*. The biopsies were performed at the same time of day on each animal. The procedure used for the analysis of the liver for vitamin A and carotene was that of Gallup and Hoefer *(8)*. Analysis of the milk fat was by the method of the technical committee in charge of the nation-wide butter survey of the U. S. Department of Agriculture *(14)*. The blood, liver, and milk fat samples were taken on the same day.

Carotene was supplied by adding definite amounts of carotene-rich oil (beta-carotene) to the ration (Table 1). Vitamin A ester[4] was injected intravenously every 5 days in prescribed dosages. During the periods the experimental animals received vitamin A they were fed a ration containing approximately 50 γ carotene per kilogram of body weight. The normal ration unless otherwise indicated consisted of grass silage and well-cured second-cutting alfalfa hay and irrigated pasture in season.

RESULTS

Table 1 gives the results of the blood, liver, and milk fat analyses corresponding to the carotene and vitamin A intakes of the cows used.

Jersey 251. This cow after nearly 5 years on low-carotene rations had blood plasma carotene and vitamin A values above the level considered to be critical for reproduction. However, the liver and milk fat values were extremely low when compared with those of normal cows (218 and 224) of the same breed. Fifty γ of carotene per kilogram of body weight in the ration of Cow 251 supplemented by a dosage of 250,000 I.U. vitamin A ester injected intravenously every 5 days for 40 days failed to appreciably increase blood, liver, and milk fat levels of vitamin A and carotene. With 50 γ of carotene per kilogram of body weight in the ration, not supplemented with vitamin A ester injections over a subsequent period of 27 days, there was a reduction in blood, liver, and milk fat values. Intravenous injection of 1,250,000 I.U. of vitamin A ester every 5 days for an

[4] Provided by Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N. Y.

TABLE 1

Carotene and vitamin A values of blood plasma, liver and milk fat of cows with variable carotene intakes and vitamin A injections

Cow	Date	Blood plasma		Fresh liver		Milk fat		Carotene intake	Vitamin A injection every 5 days
		Carotene	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A		
		(p.p.m.)	*(p.p.m.)*	*(γ/g)*	*(γ/g)*	*(γ/g)*	*(γ/g)*	*(γ/kg)*	
251	11-18-53	1.35	0.06	1.57	9.15	0.60	1.58	130	
Jersey	12-18-53	1.75	0.23	1.05	8.91	0.60	2.00	50	
	1-28-54	1.61	0.33	3.45	11.50	0.40	2.56	50	250,000
	2-24-54	0.96	0.21	0.65	6.52	0.20	1.82	50	
	3-22-54	0.67	0.29	0.70	7.25	0.10	2.18	50	1,250,000
	5-17-54	1.68	0.26	0.70	11.90	0.30	1.82	50	1,250,000
267	11-28-53	7.20	1.04	13.60	70.6	4.40	3.96	Normal[a, b]	
Jersey	12-18-53	2.66	0.53	5.00	67.5	0.80	3.34	50	
	1-28-54	1.11	0.37	9.45	43.8	0.80	3.22	50	250,000
	2-24-54	0.60	0.33	1.63	25.2	1.00	3.62	50	
	3-22-54	0.24	0.23	0.96	21.8	0.20	3.04	50	1,250,000
	5-17-54	1.34	0.30	0.83	18.7	Dry		50	1,250,000
553	12-18-53	2.86	0.31	1.25	112.5	0.40	5.28	Normal[a, c]	
Holstein	2-15-54	1.85	0.30	1.41	44.7	0.40	4.24	130	
	3-15-54	1.44	0.25	0.63	63.5	1.00	5.60	130	
	5-24-54	1.52	0.20	0.43	22.8	0.25	2.86	130	
557	11-13-53	—	—	1.38	15.15			50[d]	
Holstein	12-18-53	3.10	0.26	1.25	24.0	1.00	3.16	390	
	2-15-54	4.80	0.31	6.50	40.6	1.00	3.32	390	
	3-15-54	3.64	0.41	3.48	34.9	1.00	4.56	390	
	5-24-54	3.10	0.30	1.54	27.4	0.70	3.52	390	
224	11-18-53	6.28	0.46	16.20	201.0	3.80	6.82	Normal[a]	
Jersey	12-18-53	2.33	0.29	7.04	182.0	1.40	4.52	50	
	1-28-54	2.50	0.31	17.55	141.0	Dry		50	250,000
	2-24-54	1.32	0.20	4.45	120.0	Dry		50	
	3-22-54	0.65	0.21	3.58	92.7	Dry		50	1,250,000
	5-17-54	5.05	0.19	8.22	98.4	Dry		50	1,250,000
218	3-8-54	8.18	0.40	12.20	134.1	7.60	6.64	Normal[a]	
Jersey	3-29-54	7.75	0.19	8.60	149.0	8.00	7.40	Normal	
553	3-29-54	8.35	0.12	9.17	162.0	2.60	7.78	Silage	
Holstein	5-17-54	12.95	0.19	16.70	183.0	4.00	9.50	Pasture	
557	3-29-54	5.65	0.22	5.32	52.7	1.40	5.56	Hay	
Holstein	5-17-54	8.20	0.38	9.82	74.0	1.80	5.02	Pasture	

[a] Alfalfa hay and grass silage.

[b] 50 γ per kilogram for 3½ years. Normal previous 6 months.

[c] 50 γ per kilogram for 3½ years. Normal previous 16 months.

[d] 50 γ per kilogram for 5 years. Liver biopsy second day after parturition.

additional 25-day period resulted in a slight increase in blood, liver, and milk fat levels of carotene and vitamin A. Continuation of the same ration and treatment for another 55 days, however, resulted in only inconsequential increases in plasma carotene and liver vitamin A. This indicates that vitamin A supplementation by intravenous injection has a mildly beneficial effect in depleted animals, but not in proportion to the amount injected. Furthermore, it shows that 50 γ of carotene per kilogram of body weight in the ration is insufficient to maintain blood, liver, and milk fat levels of carotene and vitamin A.

Jersey 267. This animal was raised from birth to 3½ years of age on a low-carotene ration and after her second calving was placed on a normal ration of irrigated pasture and grain. After she had been on the normal ration for 6 months the blood plasma carotene and vitamin A values were normal, the liver vitamin A value was low, and the milk fat had a normal carotene value, but the vitamin A value was somewhat low. After 20 days of a carotene intake of approximately 50 γ per kilogram of body weight all values had dropped, with the least drop showing in the liver and milk fat vitamin A values. Intravenous injection every 5 days of 250,000 I.U. of vitamin A ester for 40 days failed to raise or maintain the blood and liver carotene and vitamin A values, and the milk fat values remained essentially the same.

When Cow 267 was returned to a carotene intake of 50 γ per kilogram of body weight but with no vitamin A injections for 27 days, the blood and liver carotene and vitamin A values decreased, but the milk fat values were not appreciably influenced. The blood and liver values continued to decrease even when the cow was receiving the low-carotene hay plus 1,250,000 I.U. of vitamin A ester intravenously every 5 days. The carotene level in the milk fat dropped considerably, but the vitamin A value of the milk fat decreased only a small amount. After 80 days of this treatment the liver values still were lower, but the blood values had increased slightly. These data suggest that the intravenous injections of 250,000 or 1,250,000 I.U. of vitamin A ester every 5 days are not sufficient to maintain or raise the liver vitamin A values of cows that have been maintained for an extended period of time on a suboptimal intake of carotene and whose livers contain minimal amounts of vitamin A.

Holstein 553. This animal had been raised until first calving at 3½ years on the low-carotene ration of first 50 γ and then 90 γ of carotene per kilogram of body weight. Because of severe edema of the legs this cow was placed on a normal ration immediately after her first calving. The first liver biopsy was made after 1 year on the normal ration and following the second calving. At this sampling the carotene and vitamin A values for the blood, liver, and milk fat fell within normal range. Fifty-nine days after being returned to a low-carotene ration of 130 γ of carotene per kilogram of body weight, the vitamin A value of the liver had dropped considerably, although the plasma and milk fat values remained essentially the same. Two subsequent sampling periods showed a continued drop in the liver but no change in the blood and milk fat until the last sample, which showed a considerable drop in the carotene and vitamin A value of the milk fat. The information on Cow 553 suggests that 130 γ of carotene per kilogram of body

weight is not sufficient to maintain the blood and liver carotene and vitamin A values of a lactating cow and that the cow draws on the liver stores of vitamin A to maintain the vitamin A milk fat value.

Holstein 557. This cow, nearly 5 years old, had been raised first on 50 γ and then 90 γ and at the time of her second calving was receiving 130 γ of carotene per kilogram of body weight. The first liver biopsy sample was taken the second day after parturition and showed low carotene and vitamin A values. The low-carotene hay was continued, but a carrot oil supplement was fed in such quantity that the cow was receiving 390 γ of carotene per kilogram of body weight. Four subsequent samples of the blood, liver, and milk fat showed little change in the carotene and vitamin A values, indicating that in Cow 557, raised and maintained on a low-carotene ration, 390 γ per kilogram of body weight of carotene was not sufficient to raise the carotene and vitamin A values of the blood, liver, and milk fat to what might be called normal levels.

Jersey 224. This cow was 7 years old and had been raised and maintained on a normal ration. First sampling indicated that the carotene and vitamin A values of the blood, liver, and milk fat were in what might be called a normal range. When the cow was fed a low-carotene ration of approximately 50 γ of carotene per kilogram of body weight, the values all dropped considerably. Feeding the low-carotene ration and injecting intravenously every 5 days either 250,000 or 1,250,000 I.U. of vitamin A failed to prevent the lowering of the blood and liver carotene and vitamin A values. However, when the animal was placed on the normal ration and the injections of 250,000 I.U. of vitamin A were continued, the blood and liver carotene and liver vitamin A values began to increase slightly. These data further suggest that 250,000 I.U. of vitamin A ester when given to a dairy cow on a low-carotene hay is insufficient to maintain the carotene and vitamin A levels of the blood and liver.

Jersey 218. This animal was raised and maintained on a normal ration, and samples of the blood, liver, and milk fat indicate values for carotene and vitamin A that can be assumed to be normal. However, the blood plasma vitamin A was a poor indicator of carotene intake.

Holstein 533. Cow 533 was raised and maintained on a normal ration with good quality grass legume silage and grain being fed for 6 weeks prior to taking the first sample. The second sample was taken after the cow had been on irrigated pasture for 3 weeks. The carotene and vitamin A values at both samplings are within the normal range for mature cattle. Again, the blood vitamin A was not related to the carotene intake.

Holstein 577. The animal had been maintained on a normal ration, which for the 6 weeks prior to taking the first sample consisted solely of high quality alfalfa hay and grain. The liver vitamin A value in this cow was low, but not in the depleted state of the low-carotene animals. The second sample from Cow 577, taken after she had grazed on irrigated grass and Ladino clover pasture for 3 weeks, showed an appreciable rise in the vitamin A and carotene values of the liver.

The data on Cows 533 and 577 suggest that alfalfa hay is not as adequate as grass silage in maintaining liver vitamin A, although the difference could be due to age, since Cow 533 is approximately 2 years older than Cow 577.

DISCUSSION

This study has shown that animals of the Jersey and Holstein breeds from dams fed low-carotene rations in the latter part of their pregnancies and which have been maintained for nearly 5 years at 50, 90, and 130 γ of carotene per kilogram of body weight have extremely low liver carotene and vitamin A values. Although information on normal values for mature dairy cattle is fragmentary, it would seem reasonable to describe the livers of the low-carotene animals as being at depletion levels, inasmuch as the data presented in this study indicate a range from about 75 to 200 γ of vitamin A per gram of fresh liver for normal cows.

Repeated injections every 5 days of either 250,000 or 1,250,000 I.U. of vitamin A ester failed to result in any appreciable increase of liver vitamin A in normal or depleted animals. This result is surprising.

Evidence is presented which indicates that a cow beginning with normal liver values may maintain the milk fat values at the expense of the liver when she is receiving 130 γ of carotene per kilogram of body weight. It should be noted, however, that Cow 553 was raised to an age of 3½ years on the low-carotene ration before being placed on the normal ration. It would also appear that 390 γ of carotene per kilogram of body weight will not increase the liver vitamin A to normal values in depleted animals.

The study indicates that in normal Holstein cows alfalfa hay does not maintain the liver vitamin A value as well as does grass-legume silage and that neither maintains it as well as does irrigated pasture. That the liver continues to store vitamin A when a cow goes from alfalfa hay or grass silage to pasture may mean a physiological need beyond what we have heretofore considered merely a matter of frugal storage.

The possibility of liver storage of vitamin A being limited by previous nutrition is indicated. The capacity of the liver to store vitamin A may possibly have been affected by the previous extended feeding (prenatal and postnatal) of low-carotene rations.

This study shows that additional information on the requirements, utilization, conversion, and storage of carotene and/or vitamin A in dairy cattle is necessary.

SUMMARY

Blood plasma, liver, and milk fat analyses for carotene and vitamin A in Jersey and Holstein cows on normal and suboptimal carotene rations were compared. Suboptimal carotene rations fed prenatally and over long periods of time after birth resulted in low liver vitamin A and carotene values, which failed to respond to carotene supplementation as high as 330 γ per kilogram of body weight. Repeated injections every 5 days of either 250,000 or 1,250,000 I.U. of vitamin A ester failed to result in any appreciable increase of liver vitamin A or carotene in cows on a suboptimal carotene ration.

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A COMPARISON OF AMMONIATED MOLASSES, UREA, AND COTTONSEED MEAL AS A SOURCE OF NITROGEN IN THE RATION OF DAIRY HEIFERS

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There has been a sharp increase in the demand for protein supplements in the past few years. This is largely due to the increase in the number of livestock, but it can also be attributed to a greater utilization of poor quality roughages, adequately supplemented, in wintering and fattening rations. It has been established that the rumen microflora can convert nonprotein nitrogen to protein, which makes possible the utilization of various protein substitutes.

In 1939, Hart *et al. (6)* conducted the first long-time growth studies with nonprotein nitrogen for ruminants. Calves fed an ammonium bicarbonate ration gained 1 lb. per day as compared to a gain of 1.5 lb. per day by the calves on a casein ration. Millar *(13)* found that when the crude protein of a ration (6.94%) was increased to 17% by replacing plain beet pulp with ammoniated beet pulp the calves made normal gains. Other workers *(7, 8, 11, 12, 19)* concluded that ammoniated products, fed at a 10% level, did not significantly affect either the body weight of calves over 12 weeks of age or milk production of dairy cows.

Since urea is soluble in molasses, combinations of urea and molasses may be made for any desired protein equivalent *(3)*. Loosli *et al. (9, 10)* fed young calves a ration containing 4% urea as soon as they would consume dry feed. The calves continued to grow at a fairly normal rate after the milk was removed from the diet at 7 weeks of age. In a preliminary feeding trial conducted by Ratcliff and Stallcup *(17)* urea was used to replace one-third of the protein of cottonseed meal in the grain mixture for dairy heifers 5 to 11 months of age. The urea-fed heifers gained slightly more than the control heifers. It has been reported that urea nitrogen is not as efficient as nitrogen from plant sources for the production of milk *(18, 21, 22)*.

This experiment was conducted to determine the value of ammoniated molasses (32%) and urea (262%) as a substitute for cottonseed meal for growing dairy heifers 19 to 33 months of age.

EXPERIMENTAL PROCEDURE

The experimental design consisted of three rations and three groups of dairy heifers, each group being fed each ration for a period of 28 days, with a 7-day change-over period. Thirty-three purebred dairy heifers (24 Holsteins and nine Jerseys) were grouped into three comparable lots. The heifers were fed individually, in dry lots, 25 lb. daily of corn and soybean silage, 3 lb. of chopped grass hay, and grain according to body weight, averaging 0.9 lb. per 100 lb. of body weight. The composition of the concentrate mixtures is presented in Table

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TABLE 1
Composition of concentrate rations

Constituents	Ammoniated molasses	Urea	Cottonseed meal
	(%)	*(%)*	*(%)*
Oats	30.00	30.00	30.00
Corn	55.50	54.05	44.50
Ammoniated molasses	12.00	—	—
Urea	—	1.45	—
Cottonseed meal	—	—	11.00
Cane molasses	—	12.00	12.00
Steam bone meal	1.50	1.50	1.50
Salt	1.00	1.00	1.00

1. All uneaten feed for each animal was weighed back daily. The animals were weighed at the beginning and end of each period for three consecutive days. The concentrate mixtures were formulated to contain approximately 12.7% crude protein, in which the ammoniated molasses and urea replaced 30% of the protein equivalent. Chemical analyses were made of concentrates and forages according to A.O.A.C. methods *(1)*, and the energy values were obtained with an Emerson bomb calorimeter. Statistical analysis of the data was made according to the method of Snedecor *(20)*.

RESULTS AND DISCUSSION

The chemical composition of the rations is presented in Table 2. The ammoniated molasses ration was slightly higher in protein content than the other rations. This is due to the fact that the ammoniated molasses contained slightly more than 32% protein equivalent; however, the rations were isocaloric.

TABLE 2
Chemical analysis of concentrates and forages (dry matter basis)

Feed	Moisture	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Energy
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(Cal/g)*
Ammoniated molasses mixture	10.7	13.1	2.7	3.3	62.8	6.3	4.03
Urea mixture	11.7	12.6	2.9	3.6	63.2	5.9	3.86
Cottonseed meal mixture	10.5	12.4	3.1	3.9	62.3	6.5	3.87
Corn & soybean silage	72.4	2.9	0.5	9.5	12.0	2.7	—
Grass hay	10.4	8.0	1.2	30.7	39.9	9.8	—

During the preliminary period of approximately 1 month, most of the heifers failed to maintain their weight when fed according to Morrison's standards recommended for growing dairy heifers *(16)*. The rations were altered to provide 0.9 lb. of the concentrate mixtures per 100 lb. of body weight. The data summarizing the effect of treatment on growth rates and feed utilization appear in Table 3. Group 1, during Period I, made lower daily gains than the other groups, but the differences were not significant at the 5% level (Table 3). The daily gains were more satisfactory during the second and third periods. The

TABLE 3

Growth gains and feed utilization of dairy heifers

	Group I[a]			Group II			Group III		
	Period 1[b]	Period 2	Period 3	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
Av. wt. per heifer *(lb.)*									
Initial	797.0	816.0	866.0	804.0	838.0	877.0	795.0	820.0	865.0
Final	810.0	852.0	904.0	832.0	869.0	912.0	816.0	852.0	905.0
Daily gain	0.47	1.31	1.35	1.00	1.12	1.24	0.73	1.13	1.43
Av. feed consumed daily *(lb.)*									
Hay	2.8	2.8	2.9	2.8	2.8	2.9	2.8	2.8	2.9
Silage	23.6	24.0	24.8	23.6	23.4	24.1	23.6	23.7	24.3
Concentrates	6.94	7.20	7.40	7.05	7.34	7.47	6.92	7.10	7.32
Feed required per lb. gain *(lb.)*									
Ammoniated mol. ration			5.6		6.7		9.7		
Urea ration	15.1					6.1		6.4	
Cottonseed meal ration		5.6		7.2					5.2

[a] Each group contained 11 heifers (8 Holsteins and 3 Jerseys).

[b] Each period consisted of 28 days.

average daily gains for the three periods on the ammoniated molasses, urea, and cottonseed meal were 1.04, 0.92, and 1.23 lb., respectively. An average of 7.3, 9.2, and 6.0 lb. of feed per pound of gain was required on the ammoniated molasses, urea, and cottonseed meal rations, respectively. These differences may be due to the utilization of the nitrogen compounds in the rations, since they were isocaloric. It is noted from Figure 1 that the different rations seemed to

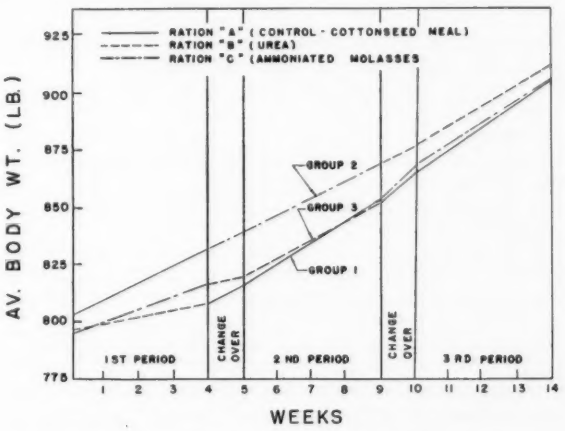

FIG. 1. Growth trends of animals on three different rations.

have little or no effect on the growth trend of the animals. The kind of carbohydrates fed may account for this response, since several investigators have reported *(2, 4, 5, 14, 15)* that cereal grains promoted greater nitrogen retention and greatly increased the utilization of urea by ruminants.

SUMMARY

A feeding trial, involving 33 dairy heifers, was conducted to study the effect of ammoniated molasses and urea in place of cottonseed meal in the regular ration of dairy heifers. Ammoniated molasses and urea supplied 30% of the protein equivalent in the concentrate and 18% of the protein equivalent in the total ration. Similar gains were made by all groups receiving either ration. The average daily gains for the heifers on the ammoniated molasses, urea, and cottonseed meal rations were 1.04, 0.92, and 1.23 lb., respectively. More feed or energy was required to produce a unit of gain by the animals receiving urea. Neither ammoniated molasses nor urea altered the palatability of the feed. No physiological effects due to the feeding of ammoniated molasses or urea were observed in any of the animals receiving these supplements in their ration.

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EXCRETION OF HEPTACHLOR EPOXIDE IN THE MILK OF DAIRY COWS FED HEPTACHLOR-SPRAYED FORAGE AND TECHNICAL HEPTACHLOR

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Heptachlor [1 (or 3a), 4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene] fed to dogs and rats has been shown to be metabolically altered and stored as the epoxide derivative in the fat of these animals *(2, 4)*. The epoxide metabolite has also been found in the milk of a dairy cow fed heptachlor *(3)*. Heptachlor has been found to be an effective insecticide for the control of such economically important insects attacking forage as the alfalfa weevil and the meadow spittlebug.

The objectives of this investigation were *(a)* to determine if heptachlor or its epoxide metabolite was excreted in the milk of dairy cows fed hay that had been previously sprayed in the field with heptachlor, and *(b)* to study the excretion of heptachlor or heptachlor epoxide in the milk of the same cows fed various intakes of technical heptachlor with untreated hay following the insecticide-treated hay feeding.

EXPERIMENTAL PROCEDURE

Two similar plots of alfalfa of approximately 4 acres each were sprayed with heptachlor emulsion at the rate of 3.8 and 8.0 oz. per acre on July 7, 1952. The insecticide was applied with a sprayer mounted on a small truck equipped with a specially designed speedometer to aid in securing uniform coverage. The sprayer delivered approximately 6 gal. per acre at a pressure of 40 p.s.i. The hay on both plots was cut 7 days after spraying and was field-cured, field-baled, and stored until fed.

Four normal, healthy milking cows (three Jersey, one Holstein) were fed ad libitum on the hay sprayed with 3.8 oz. of heptachlor per acre from Nov. 15, 1952, to Jan. 5, 1953. The hay sprayed with 8.0 oz. of heptachlor per acre was fed to the same cows from Jan. 6 to Feb. 27, 1953.

After the two hay-feeding periods the same cows were fed individual dosages of technical heptachlor dissolved in soybean oil for a 50-day feeding period. Each animal was then fed an increased intake of heptachlor in soybean oil for an additional 70-day feeding period. The heptachlor in soybean oil was administered

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by capsule twice daily, and a control hay containing no insecticide residue was fed. The sequence of the four feeding periods is outlined below.

Period I——54 days—heptachlor-sprayed hay. (3.8 oz/acre)
Period II——52 days—heptachlor-sprayed hay. (8.0 oz/acre)
Period III—50 days—heptachlor in soybean oil.
Period IV[1]—70 days—heptachlor in soybean oil.

All animals were fed sufficient hay to satisfy their appetites and provide a 10 to 15% roughage refusal. Varying amounts of a milking herd grain ration were fed, depending upon the level of milk production. Individual daily records were kept of the amount of feed fed and refused and the amount of milk produced. Each animal was weighed on two successive days at 10-day intervals. Samples of hay were taken from several locations in each bale fed and were composited for analyses for each 10-day feeding period. An aliquot of the milk produced by each animal for 2 days during each 10-day period was used for insecticide and butterfat analyses. The butterfat was separated from the milk by the method of Sager and Sanders *(5)*. Heptachlor epoxide concentrations in the butterfat were determined by the method of Radomski and Davidow *(4)*. The results were calculated to the equivalent concentration of heptachlor epoxide in fat-corrected milk (FCM).

Heptachlor residues on the hay were calculated from the organic chlorine content (corrected for the organic chlorine content of control hay) determined by the method of Carter and Hubanks *(1)*.

RESULTS AND DISCUSSION

A summary of the results of feeding the two lots of heptachlor-sprayed alfalfa hay is presented in Table 1. The hays sprayed with 3.8 and 8.0 oz. of heptachlor per acre had average insecticide residues of 1.2 and 5.0 p.p.m., respectively. The average daily heptachlor intake was 0.03 mg. per kilogram of body weight or from 8.7 to 14.0 mg. when feeding the hay sprayed with 3.8 oz. of heptachlor per acre.

The average daily heptachlor intake of the animals fed the hay sprayed with 8.0 oz. of heptachlor per acre ranged from 0.10 to 0.13 mg. per kilogram of body weight or a total intake of from 31.9 to 57.7 mg. No heptachlor or heptachlor epoxide was found in the milk of these cows while feeding either of the insecticide-sprayed forages. Daily observations of the animals for hyperirritability, muscular twitching, inappetence, and decreased milk production were negative, indicating that there were no toxic effects on the animals from eating the heptachlor-sprayed hays.

A summary of the results of feeding various dosages of technical heptachlor in soybean oil is given in Table 2. Daily intakes ranging from 100 to 1,800 mg. were fed for 50 or 70 days. These intakes were equivalent to 8.7 to 125.0 p.p.m.

[1] Three additional cows (N191, N193, N194) that had not previously received heptachlor were fed heptachlor in soybean oil at a later date and included in this group.

TABLE 1

Summary of heptachlor-sprayed hay feeding experiment

Animal	Feeding period	Av. daily hay consumed	Heptachlor on hay			Av. daily FCM	Heptachlor epoxide in FCM
	(days)	*(lb.)*	*(p.p.m.)*	*(mg/kg)*[a]	*(mg. daily)*	*(lb.)*	*(p.p.m.)*
N638	54	20.4	1.2	0.03	11.1	10.6	0
N680	54	16.0	1.2	0.03	8.7	16.1	0
N681	54	17.8	1.2	0.03	9.7	13.3	0
N805	54	25.8	1.2	0.03	14.0	30.0	0
N638[b]	20	18.9	5.0	0.11	43.2	4.1	0
N680	52	13.5	5.0	0.11	32.0	12.9	0
N681	52	14.0	5.0	0.10	31.9	11.2	0
N805	52	25.3	5.0	0.13	57.7	27.4	0
N667	30	16.5	5.0	0.11	37.6	23.9	0

[a] Milligrams of heptachlor daily per kilogram of body weight.
[b] N638 was replaced by N667 after 20 days of feeding in Period II.

of the ration (hay plus grain), and to 0.30 to 3.78 mg. per kilogram of body weight.

No heptachlor or heptachlor epoxide was detected in the milk of cows fed less than 500 mg. of heptachlor daily. Increasing concentrations of heptachlor epoxide in the milk were observed when heptachlor intakes of more than 1.6 mg. per kilogram of body weight were fed. When approximately 3.0 mg. of heptachlor daily per kilogram of body weight was fed, the concentration of heptachlor epoxide in the milk was approximately the same as found by Davidow *et al. (3)* when feeding a comparable intake.

When daily intakes of heptachlor were high enough to detect heptachlor epoxide in the milk of cows fed technical heptachlor, they excreted from 0.3 to 2.8% of the heptachlor fed as the epoxide metabolite. No symptoms of toxicity were observed in any of the animals fed technical heptachlor in soybean oil in this experiment.

The results of this experiment indicate that forage harvested at least 1 week

TABLE 2

The excretion of heptachlor epoxide in the milk of cows fed heptachlor in soybean oil

Animal	Feeding period	Heptachlor fed daily			Av. daily FCM	Heptachlor epoxide in FCM		Dose excreted
	(days)	*(mg.)*	*(p.p.m.)*[a]	*(mg/kg)*[b]	*(lb.)*	*(p.p.m.)*	*(mg. daily)*	*(%)*
N667	50	100	28.7	0.30	26.6	0.0		
N680	50	200	20.6	0.66	17.4	0.0		
N191	70	300	20.2	0.60	24.9	0.0		
N681	50	500	55.6	1.60	15.5	0.2	1.3	0.3
N193	70	600	44.6	1.30	29.8	0.2	2.9	0.5
N667	70	800	71.4	2.34	21.7	0.8	8.1	1.0
N194	70	900	53.0	1.95	34.6	0.4	6.5	0.7
N680	70	900	91.4	2.93	14.5	1.1	7.5	0.8
N681	70	1,000	110.5	3.17	12.9	1.8	10.8	1.1
N805	50	1,000	66.2	2.12	24.7	2.8	28.5	2.8
N805	70	1,800	125.0	3.78	17.5	5.7	45.0	2.5

[a] Heptachlor intake calculated as p.p.m. of the feed consumed.
[b] Milligrams of heptachlor fed per kilogram of body weight.

after spraying with as high as 8.0 oz. of heptachlor per acre is safe for feeding livestock. This dosage is higher than normally recommended for the control of insects attacking the forage of crops that are cut for hay. Other conditions affecting the heptachlor residue on the forage would, of course, alter the margin of safety. The animals fed the hay sprayed with 8.0 oz. of heptachlor per acre had daily intakes of heptachlor that were approximately one-tenth the amounts required for the detection of heptachlor epoxide in the milk under the conditions of this experiment.

SUMMARY

No heptachlor or heptachlor epoxide was detected in the milk of cows fed hay made from forage sprayed with 3.8 to 8.0 oz. of heptachlor per acre and harvested 7 days after spraying. Heptachlor epoxide was detected in the milk of a cow fed 1.3 mg. of heptachlor per kilogram of body weight. Cows fed increasing amounts of heptachlor (up to 3.78 mg. per kilogram of body weight) had concentrations of heptachlor epoxide in the FCM as high as 5.7 p.p.m.

From 0.3 to 2.8% of the heptachlor fed was excreted in the milk as the epoxide metabolite.

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EMBRYONIC MORTALITY BETWEEN 16 AND 34 DAYS POST-BREEDING IN COWS OF LOW FERTILITY[1]

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A high incidence of embryonic mortality occurs in dairy cattle of low fertility. In a study on the nature of reproductive failures in repeat-breeder cows, Tanabe and Casida *(3)* estimated that 65.1% of fertilized ova in cows of this type had died before 34 days. Casida *(1)* reported 59.4% embryonic mortality over the same period of gestation for a greater number of repeat-breeder cows. A similar high occurrence of embryonic death (54.1%) by 30 days of gestation in repeat-breeder heifers has been reported by Tanabe and Almquist *(2)*.

It has not been determined previously whether the early embryonic death in repeat-breeders occurs at a uniform rate throughout the period before the cows return to heat or whether there is a particularly critical period for embryonic death. The purpose of this study was to determine the percentage of repeat-breeder cows with normal embryos at 16 elapsed days after the beginning of the estrus at which they were inseminated and to obtain an estimate of the embryonic mortality between 16 and 34 elapsed days in such animals.

MATERIALS AND METHODS

The repeat-breeder cows used in this study were obtained from dairymen throughout Wisconsin and east-central Minnesota between March, 1953, and August, 1954. They were selected by the authors on the following bases: *(a)* bred at least four times previously without apparent conception, *(b)* no purulent discharge or physical abnormality of the genital organs detectable by palpation per rectum, *(c)* not more than 10 years old, and *(d)* bred and returned to heat within the last 90 days. Not more than two cows were taken from any one herd.

The cows were trucked to a University farm near Madison as they were selected. Heat checks were made twice daily; a cow was considered to be in heat when she stood for mounting by another cow. All animals were artificially inseminated approximately 12 hours after estrus was detected with 1 ml. of day-of-collection semen diluted with milk and containing sulfa and antibiotics. The semen was obtained from unselected stud bulls regularly used in artificial breeding and was deposited in the body of the uterus.

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Fifty repeat-breeder cows were assigned as 34-day control animals at the time of breeding. Over the same period of time, 20 of the cows that were originally in the 34-day group but returned to heat before 34 days had elapsed, plus 30 others, were assigned to be slaughtered on the 16th day following the appearance of the heat at which they were inseminated. The 20 return cows from the 34-day group differed from the other 16-day cows in having one additional service but were included because no difference in fertilization rate was observed between two groups of repeat-breeders with four to six, and seven to 13, previous services *(3)*.

The ovaries of the 34-day cows were palpated 10 days post-estrus to determine the presence of a corpus luteum, then were palpated again on the 17th day and every 3 days thereafter to check for regression of the corpus luteum and any quiet ovulations which might occur. The cows that returned to heat were rebred and slaughtered 16 days later or assigned to another study. All of the 34-day cows which had not returned to heat or had a quiet ovulation before 34 days had elapsed were slaughtered at the end of that interval. The uterus was dissected to determine the normality of the embryo if one were present.

All except five of the 16-day animals were slaughtered at the end of 16 elapsed days from the beginning of heat; the five exceptions were slaughtered at the end of 15⅔ elapsed days. The reproductive tracts were removed soon after slaughter, and the uterine horn on the side of ovulation was dissected free and excised from the remainder of the tract at the internal bifurcation. The posterior end of the excised horn was closed with hemostatic forceps, and about 15 ml. of physiological saline was introduced into the lumen through the anterior end. The horn was inverted two or three times and the forceps were released carefully over a large watchglass. Each embryo was measured, and the embryonic disc was examined under a binocular microscope. An embryo was never found upon repeated flushings of the uterine horn when one was not recovered at the first flushing. The uterine flushings were examined for a degenerate ovum when no embryo was found.

During the course of the study, eight cows were found at the time of slaughter to have either permanent or temporary abnormalities which had not been noted previously and which would influence conception (two cases of bilateral hydrosalpinx, one of bursitis, two with a completely double cervix, and three of pyometra). These animals were excluded from the experiment and replaced by others. The study was summarized when each group was represented by 50 observations on genitally normal cows.

The total of 80 cows from which data were obtained included 45 Holsteins, 28 Guernseys, five Jerseys and two Brown Swiss. Their mean age was 5.4 years, and they had calved an average of 2.6 times. All except four of the animals had calved at least once; these four were divided equally between the 16- and 34-day groups. The fifty 34-day cows had been bred an average of 7.2 times prior to the experimental service, and the fifty 16-day cows an average of 8.0 times. The difference is due partly to the fact that 20 of the 16-day animals had been bred once as 34-day animals.

RESULTS

Fourteen (28%) of the fifty 34-day animals had normal embryos. Crown-rump measurements varied between 13 and 18 mm. A degenerating embryo was found in three cows, a hemorrhagic embryo in one cow, and degenerate embryonic membranes in another. Twenty-eight cows returned to heat before 34 days and three others had a quiet ovulation before that time.

Embryos were recovered from the uteri of 29 (58%) of the 50 cows that were slaughtered 16 days after the beginning of heat (27 singles and two pairs of twins). Two degenerate eggs still showing a zona pellucida were recovered from the other 21 cows. Apparently all of the embryos were normal if judgment is based on the appearance of their tissues and the presence of a prominent embryonic disc. If over-all size is a criterion, however, judgment is more difficult. The extra-embryonic membranes varied in length from 2 to 225 mm. and measured between 1 and 2 mm. in width. The mean length of the 31 embryos was 95 mm., but the individual lengths possibly were not distributed normally around the mean. The frequency of various lengths is as follows: six ranged between 2 and 25 mm., three were 26 to 50 mm., six—51 to 75 mm., two—76 to 100 mm., two—101 to 125 mm., four—126 to 150 mm., four—151 to 175 mm., one—176 to 200 mm., and three—201 to 225 mm.

In general the embryonic discs were slightly elongated and measured 0.4 to 0.6 mm. in length; those of the longer embryos tended to be the most elongated. The embryonic discs protruded 0.2-0.3 mm. above the surface of the membranes but the head fold was not yet apparent.

The difference between the percentages of apparently normal embryos at the two stages of gestation was statistically significant ($P < 0.01$). The percentage of normal embryos at either stage of gestation was not affected significantly by breed, age, or number of calvings of the cow, the bull stud which bred the cow in the field, or the number of previous inseminations (ranging from 4 to 15). The embryonic recovery rate in the twenty 16-day cows that had been bred once as 34-day animals and returned did not differ from that of the 30 others that had not been used previously in the study (57.1% and 58.6%, respectively).

The estimate of embryonic mortality from 16 to 34 days in these repeat-breeder cows was 51.7%, the embryonic mortality rate being calculated as the per cent of 16-day embryos that died by 34 days $\left(\frac{29-14}{29}\right)$. Actually, the majority of embryonic deaths during this period appears to occur by 25 days, since 28 (77.8%) of the 36 cows which did not have a normal embryo at 34 days had either returned to heat or had a quiet ovulation by the end of 25 days.

DISCUSSION

The fertilization rate under conditions of this study is not known for repeat-breeder cows; hence, an entirely reliable estimate of embryonic mortality during the first 16 days cannot be made. Casida *(1)* reported a fertilization rate of 60.7% in 129 genitally-normal repeat-breeder cows from which eggs were re-

covered. However, the semen used for insemination was diluted with substances other than milk and did not contain sulfa or antibiotics. In a later study, Tanabe and Almquist *(2)* reported that 66.7% of 3-day ova recovered from 63 repeat-breeder heifers were fertilized; however, if genitally normal animals are included also from which no ova were recovered the per cent of heifers having fertilized eggs was 59.1, a figure which might well be more comparable to the percentage of cows in the present study with fertilized eggs. The results of this study and those just cited indicate that the great majority of embryonic deaths occurring between 3 and 34 days in repeat-breeder cows actually occur between the end of 16 days of gestation and the day the cow returns to heat.

According to the study of Winters *et al. (4)* on prenatal development in the bovine, the 16-day embryo is in the primitive streak stage, with rapid growth of mesoderm and formation of extra-embryonic somatopleure, splanchnopleure, and coelom taking place. Development of the neural system, cardiovascular system, and various internal and sense organs begins within the next 2 or 3 days. It is not known whether death of the embryo in repeat-breeder cows shortly after the 16 days is due to maternal factors or to some predisposing factor within the embryo which becomes lethal at about the time of organogenesis. A pertinent question in this regard is whether the embryo dies before the corpus luteum regresses or whether regression of the corpus luteum causes death of the embryo.

The variation in length of 16-day embryonic membranes in normal cows is unknown. Thus, it is not possible to associate the variation in length of embryonic membranes found in this study with embryonic death, although such an association may exist. The study of Winters *et al. (4)* indicates that the end of 16 days of gestation falls during a stage of rapid elongation of the extra-embryonic membranes; hence, considerable variation in length might be expected normally at this stage.

SUMMARY

A study was made to determine the percentage of repeat-breeder cows with normal embryos at 16 elapsed days of gestation and the embryonic death rate between 16 and 34 elapsed days. Apparently normal embryos were recovered from the uteri of 29 (58%) of 50 repeat-breeder cows slaughtered 16 days after the first day of heat; 50 repeat-breeder cows at 34 days had 14 normal embryos (28%). The estimate of embryonic death from 16 to 34 days was 51.7%.

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STUDIES ON THE AVAILABILITY OF NITROGEN FROM VARIOUS AMMONIATED PRODUCTS FOR RUMEN BACTERIA AND DAIRY CATTLE

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The circumstances essential for the efficient utilization of urea nitrogen by the ruminant have been reviewed by Reid *(13)*. One serious problem concerning the utilization of urea and ammonia is their rapid disappearance from the rumen. Removal of either of these soluble nitrogenous substances from the sphere of rumen bacterial action makes them useless as sources of protein for the host animal. In an attempt to solve this problem, many industrial products and feeds have been impregnated with ammonia under varying conditions of temperature and pressure to induce chemical combinations that might release utilizable nitrogen more in proportion to the rate at which energy from complex carbohydrates becomes available. Thus, the energy-dependent protein synthetic reactions would have conditions more suitable for the conversion of nonprotein nitrogen to protein.

Tests of several of these products have been reported by different authors. MacGruder *et al. (10)* reported on the feeding of an ammoniated building by-product. Experiments with ammoniated citrus pulp, beet pulp, molasses, and other feeds have been reported by various workers *(4, 7, 11, 12, 15)*.

It has been generally concluded that nitrogen from these ammoniated compounds is available to rumen bacteria. However, some of these experiments have been conducted under conditions which do not allow a critical assessment of the availability of nitrogen from ammoniated materials. In some studies, the ammoniated products furnished either a relatively small proportion of the total nitrogen of the ration or the nitrogen in the ration was fed at or above the animal's requirement. When Ferguson and Reeves *(4)* took these factors into consideration, they found that the nitrogen in ammoniated beet pulp was relatively unavailable in comparison with urea.

In order to obtain more data on the availability of nitrogen from ammoniated products, a series of in vitro studies with rumen bacteria and a series of balance studies with dairy steers were conducted.

IN VITRO EXPERIMENTS

Procedures. A modification of the in vitro technique of Huhtanen and Gall *(5)* was employed in assessing the availability of nitrogen in ammoniated feed products. The fermentation was confined to a semipermeable sac suspended in a

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buffer-salt solution in order to decrease by dialysis the concentration of deleterious fermentation by-products *(9)*. The criteria of nitrogen availability were the amount of cellulose digestion and/or protein synthesis which occurred in the presence of limiting quantities of supplementary nitrogen in the treated products. In addition, these manifestations of rumen bacterial activity were compared with the effect of equivalent levels of ammonia-nitrogen. Urea was not employed, since high levels of urease activity are present in the rumen *(17)* and preliminary work had shown that the rapid breakdown of urea adversely affected the pH within the artificial rumen. The essential differences in the present procedure as compared to that of the aforementioned investigators are noted as follows:

(a) The artificial rumens were incubated within a desiccator made anaerobic by displacing air with CO_2; *(b)* phenol red was employed as an internal pH indicator when periodic neutralizations with IN.KOH were made during the course of fermentation; *(c)* the incubation period was usually 24 hours and never exceeded 30 hours; *(d)* the fiber substrate consisted of an alfalfa leaf meal-cellulose (Solka-Floc) mixture (1:4); *(e)* dried distillers molasses solubles were added, where noted, as a source of factors shown to be stimulatory for cellulose digestion *(16)*. The buffer-salt solution shown in Table 1 was based on the solution used by Burroughs *et al. (2)* in their artificial rumen studies.

TABLE 1

Composition of the buffer-salt solution employed in the in vitro study

	(%)		*(%)*
K_2HPO_4	0.46	$MgSO_4 \cdot 7H_2O$	0.12
KH_2PO_4	0.46	Na_2SO_4	0.07
$NaHCO_3$	0.25	$FeCl_2$	0.04
KCl	0.56	$MnCl_2$	0.012
NaCl	0.47	Methionine	0.07
$CaCl_2$	0.04		
	pH—7.2		

The ammoniated test product, containing 4.4 mg. nitrogen, 500 mg. fiber mixture, and 100 mg. dried distillers molasses solubles, was placed within the dialyzing membrane. The dialyzing membrane and contents, after insertion into a small bottle, were sterilized by autoclaving at 15 p.s.i. for 10 minutes. Buffer-salt solution (50 ml.) was added to the bottle to serve as a source of inorganic nutrients, as a buffer, and as a recipient for substances diffusing through the semipermeable membrane. Ten ml. of this buffer-salt solution was placed within the sac. The active inoculum consisted of 5 ml. of a washed heavy suspension of rumen microorganisms from a fistulated steer fed a ration of late-cut grass hay, molasses, urea, and minerals.

After the period of incubation, the residual fiber was separated from the rumen microorganisms by differential centrifugation. The cellulose in the fiber was determined by a modification of the method of Crampton and Maynard *(3)*. The bacterial protein was estimated by hydrolyzing the protein with concentrated H_2SO_4, and the subsequent determination of ammonia-nitrogen by the nesslerization technique of LePage *(8)*.

The ammoniated products, obtained from three manufacturers, had been treated with anhydrous ammonia at various temperatures and pressures to induce the chemical combinations. In certain cases the exact ammoniation procedure was not known, but inspection of the product indicated the severity of the treatment. The products are more thoroughly described in the presentation of the balance studies.

In conjunction with these and other artificial rumen studies, the microbial population was examined by standard microscopic techniques and by the growth dilution method of Huhtanen *et al. (6)*.

Results. Examination of the microbial population indicated that the centrifugation procedure did not significantly alter the numbers and types of bacteria present. However, certain changes were obvious in the fermentation liquor after incubation. The protozoal population, which was vigorous in the inoculum, was less apparent after incubation. The cultural technique revealed that a fast growing facultative streptococcus was usually 100 times more numerous, whereas the microscopic examination of gram stains showed the bacterial population to be less diverse after incubation. However, the bacteria present in the greatest number culturally post-incubation were similar to those considered the most numerous in the pre-incubation sample, and the bacteria most frequently observed microscopically were morphologically similar to a known cellulose digester previously isolated from the rumen. All of the above findings indicate that the population had changed during incubation under artificial conditions. However, significant similarities existed between the in vitro and in vivo microorganisms. This cursory bacteriological examination indicated that certain important reservations should accompany any in vitro data when extrapolating to the live animal.

The results of the comparative nitrogen availability in the ammoniated products as shown by in vitro methods are presented in Table 2. In the first two series both cellulose digestion and protein synthesis were determined, but only cellulose digestion was estimated in the third and fourth series. Belasco *(1)* indicated that cellulose digestion was a valid indication of nitrogen utilization or availability in vitro. Statistical evaluation of the data in Table 2 revealed that the correlation coefficient between the two measurements (cellulose digestion vs. protein synthesis) was 0.920 (degrees of freedom = 8), which is significant at the 0.01 probability level.

The data suggest that the procedures employed in ammoniation significantly reduce the availability of nitrogen in all of the products tested except one. This exception, Series II and IV, ammoniated furfural residue 3A, was ammoniated at atmospheric pressure followed by acid neutralization. This would indicate that the nitrogen in that particular sample was present as the ammonium ion per se or readily convertible to the ammonium ion in vitro. The other products in this fourth series were treated by high temperature, high pressure, saccharification, or combinations of these treatments. The ammoniation procedure which the citrus pulp and dried distillers molasses solubles received was unknown. It is apparent that the methods employed to introduce nitrogen into these products

TABLE 2

Effect of ammoniated by-products on cellulose digestion and protein synthesis by rumen organisms in vitro

Supplement[a]	Cellulose digestion[b]	Protein synthesis[b]
	(%)	*(mg.)*
Series I (Ammoniated citrus pulp)[c]		
Nonammoniated citrus pulp	5.4	6.0
Nonammoniated citrus pulp plus NH_4Cl	16.9	35.0
Ammoniated citrus pulp (Sample #19)	11.2	13.5
Ammoniated citrus pulp (Sample #20)	9.1	9.0
Series II (Ammoniated furfural residue)[c]		
Control	10.1	4.4
NH_4Cl	23.6	34.4
Ammoniated furfural residue #3A	24.8	38.1
Ammoniated furfural residue "A"	16.6	18.1
Ammoniated furfural residue "B"	15.5	24.4
Series III (Ammoniated dried distillers molasses solubles)[d]		
Nonammoniated DDMS	11.9	
Nonammoniated DDMS plus NH_4Cl	32.0	
Ammoniated DDMS #1	10.7	
Ammoniated DDMS #3	14.5	
Ammoniated DDMS #4	8.4	
Ammoniated DDMS #5	11.9	
Ammoniated DDMS #6	8.0	
Series IV (Ammoniated furfural residue)[c]		
Control	11.0	
NH_4Cl	21.9	
Ammoniated furfural residue 1A	9.0	
Ammoniated furfural residue 2A	10.1	
Ammoniated furfural residue 3A	20.6	
Ammoniated furfural residue 4A	13.2	
Ammoniated furfural residue 5A	13.8	
Ammoniated furfural residue 6A	14.6	

[a] The supplemental ammoniated by-products or NH_4Cl contributed 4.4 mg. *N* to each fermentation apparatus.

[b] Each value represents the mean of duplicate samples from two experiments.

[c] Basal: alfalfa leaf meal — cellulose mixture (0.5 g.) plus dried distillers molasses solubles (0.1 g.).

[d] Basal: alfalfa leaf meal — cellulose mixture (0.5 g.).

were sufficiently drastic (with the exception noted) to depress its availability to rumen bacteria as compared to ammonium chloride.

Variations also existed among these products in the availability of their nitrogen. Some products showed an increased cellulose digestion and/or protein synthesis above the negative control whereas others provided no available nitrogen. The possibility of inhibitory substances present in any of these products was not eliminated during the course of this investigation.

BALANCE AND DIGESTION STUDIES

Procedure. Three balance studies, all conducted in a similar manner, were used to determine the availability of nitrogen from several different sources of ammoniated products. Four dairy steers, ranging in weight from 400 to 550 lb., were used in each experiment. The steers in each replicate were closely matched.

Experimental periods consisted of a minimum of 1 week preliminary feeding and 1 week during which total collection of feces and urine was made. The design of the experiment was a 4 × 4 Latin square. Animals were fed their preliminary rations in individual pens; collections were made in crates especially designed for the separation and quantitative collection of urine and feces. Aliquot samples of all urine and feces were taken daily and composited. These were stored in closed containers under toluene and refrigerated until the end of the collection period, at which time samples were taken for proximate analysis. Feces samples were dried at approximately 60° C. for a minimum of 48 hours and allowed to come to equilibrium with the moisture in the atmosphere for a minimum of 48 hours before being ground in a Wiley mill and placed in sealed containers for analysis. All chemical analyses were conducted by accepted procedures.

Trial 1 was conducted with three different ammoniated residues from the manufacture of furfural. Original materials used in this process include corn cobs, oat hulls, cottonseed hulls, and other fibrous substances. Residue A had been hydrolyzed for ½ hour after sulfuric acid extraction to produce furfural, residue B had been hydrolyzed for 1½ hours, and residue C for 3½ hours. The experiment was designed to determine whether the different treatments affected

TABLE 3

Rations used in studying the value of three ammoniated furfural residues as sources of nitrogen for ruminants

Ingredient	Ration A	Ration B	Ration C	Ration D
	(%)	*(%)*	*(%)*	*(%)*
Product A	23.0	—	—	—
Product B	—	23.0	—	—
Product C	—	—	23.0	—
Soybean oil meal	—	—	—	23.0
Corn meal	35.0	35.0	35.0	35.0
Molasses	15.0	15.0	15.0	15.0
Hay	25.0	25.0	25.0	25.0
Bone meal	1.0	1.0	1.0	1.0
Salt	1.0	1.0	1.0	1.0
	100.0	100.0	100.0	100.0

the availability of nitrogen from these products in comparison with the availability of nitrogen from soybean oil meal. The residue products were finely divided powders which were very light and dusty. The composition of the rations fed is shown in Table 3. The rations were prepared by mixing the corn meal, molasses, minerals, and ammoniated product or soybean meal in a small mechanical feed mixer. The hay, which was late-cut, low-protein timothy, was chopped to 1- to 2-in. lengths and mixed with the rest of the ration at the time of feeding. All animals received the same amount of feed during each period. In addition to the minerals listed, each animal received 50 g. of monosodium phosphate (NaH_2PO_4) daily after the first period. These rations were fed at a level which more than met the nitrogen requirement of the animals in the study.

TABLE 4

Daily ration ingredients used in studying the value of ammoniated molasses and 2 ammoniated furfural residues as sources of nitrogen for ruminants

Ingredient[a]	Ration			
	Soybean oil meal	NH_4 molasses	Furameal No. 10	Furameal No. 11
	(g.)	*(g.)*	*(g.)*	*(g.)*
Hay	2268	2268	2268	2268
Corn meal	898	1452	90	0
Molasses	1315	0	1088	1043
Ammoniated molasses	0	1315	0	0
Soybean oil meal	500	0	0	0
Furameal mix No. 10	0	0	1588	0
Furameal mix No. 11	0	0	0	1906
Total	4981	5035	5034	5217

[a] Also fed were: salt, free choice; dicalcium phosphate, 50 g/day; cobalt, 0.3 mg/day; vitamin A supplement, twice weekly (Nopeay "10" supplied by Nopco Chemical Co., Harrison, N. J.).

In Trial 2 ammoniated molasses and soybean meal were employed, in addition to two products from the residues of furfural production. Residue No. 1 was a furfural by-product ammoniated at pressures greater than atmosphere. Residue No. 2 was the same material ammoniated at atmospheric pressures. The ammoniated molasses treatment was not known. The objectives of this study were the same as in Trial 1, that is, to determine the relative availability of nitrogen from these sources as compared to soybean oil meal. Furfural residues were fed in a pelleted mixture containing approximately 53% of the residue, 13% molasses, 32% corn meal, and 2% minerals. Equal amounts of hay and molasses were included in all rations. The daily rations consumed are reported in Table 4. The nitrogen content of all rations was adjusted to the lower level of Morrison's recommendations. Since the nutrient content of the residues was unknown, no attempt was made to adjust TDN intake. However, the TDN content of the control ration fell within the range of Morrison's recommendations.

TABLE 5

Daily ration ingredients used in studying the value of ammoniated DDMS and urea as sources of nitrogen for ruminants

	Rations			
	I Control	II NH_4 DDMS	III DDMS	IV Alfalfa
	(g.)	*(g.)*	*(g.)*	*(g.)*
Timothy hay	2722	2722	2722	2268
Alfalfa hay	0	0	0	454
Corn meal	1769	1315	1733	1783
DDMS	0	0	36	0
NH_4 DDMS	0	499	0	0
Urea	45	0	45	31
Total	4536	4536	4536	4536

TABLE 6

Chemical composition of the feed ingredients used in three balance studies involving ammoniated products

	Water	Nitrogen[a]	Crude protein	Ether extract	Crude fiber	Ash	Water, acid, and base soluble matter[b]
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
			Trial I				
Residue A	20.8	6.0	—	1.9	45.6	3.7	22.0
Residue B	19.4	6.6	—	2.4	51.3	3.8	16.5
Residue C	17.0	6.3	—	2.2	54.1	3.9	16.5
Hay	9.6		4.8	2.0	33.0	3.6	47.0
Corn meal	12.0		8.4	3.4	2.1	1.4	72.7
Soybean oil meal	9.5		45.9	3.4	6.7	5.7	28.8
Molasses	32.3		3.5	—	—	7.6	56.6
			Trial II				
Residue mixture 1	12.2		18.7	1.9	17.2	6.1	43.9
Residue mixture 2	11.2		16.2	2.0	18.0	6.5	46.1
Ammoniated molasses	32.8	2.6	16.3	—	—	6.5	44.4
Molasses	28.9		3.5	—	—	6.6	61.0
Hay	8.8		6.5	2.0	38.6	3.4	40.7
Corn meal	12.9		9.0	4.6	2.2	1.7	69.6
Soybean oil meal	11.6		44.3	1.4	6.8	5.4	30.5
			Trial III				
Control mix	10.5		15.9	2.9	2.0	1.6	67.1
NH_4 DDMS mix	10.9		15.1	1.7	1.9	11.3	59.1
DDMS mix	11.1		16.2	3.0	2.0	5.9	61.8
Mix fed with alfalfa	11.6		13.9	3.9	2.2	1.4	67.0
Timothy hay	7.6		5.5	2.1	35.2	3.0	46.6
Alfalfa hay	10.2		18.4	1.9	25.5	5.5	38.5

[a] Nitrogen is reported as such in the ammoniated products, since it is doubtful if there is more than a very small amount of protein material.

[b] Reported in this manner since the term nitrogen-free extract probably does not apply to the ammoniated products. This column includes nitrogen-free extract for the natural products.

Trial 3 was a similar study, in which nitrogen from ammoniated dried distillers molasses solubles (DDMS) was compared with nitrogen from urea in rations which contained the nonammoniated DDMS and a small amount of alfalfa. Previous studies indicated that DDMS had a stimulatory effect on the digestion of cellulose and the utilization of nonprotein nitrogen in vitro. These materials were included in this study in order to determine whether this effect could be demonstrated with animals. The rations fed in this experiment are reported in Table 5. Chemical compositions of all the feed ingredients are included in Table 6.

Results. Digestion coefficients obtained in all three trials are reported in Table 7. It will be noted that, compared with the soybean oil meal (control) ration, the rations which included the three different furfural residues exhibited a greatly lowered digestibility of dry matter. The digestibility of nitrogen was severely decreased when the residue products were the only source of supplemental nitrogen. Crude fiber also was digested at a much lower rate from the samples representing the furfural residue. This may be explained by the fact that this furfural residue had been already severely treated with sulfuric acid,

TABLE 7

Average digestion coefficients obtained from rations fed in three balance studies involving ammoniated products

Ration characteristics	Dry matter	Nitrogen	Ether extract	Crude fiber	Water, acid, and base soluble material[a]
	(%)	(%)	(%)	(%)	(%)
		Trial I			
Residue A	62	51	69	30	74
Residue B	60	52	73	31	74
Residue C	62	47	72	27	75
Soybean oil meal	75	66	77	54	80
		Trial II			
Residue I	60[b]	42[b]	65	41[b]	73
Residue II	61[b]	43[b]	66	43	74
Ammoniated molasses	64	45[b]	70	47	75
Soybean oil meal	67	56	69	49	78
		Trial III			
Corn and urea	63	64	69	47	71
Corn and ammoniated DDMS	60	45	71	48	68
Corn, urea, and DDMS	64	65	76	50	71
Corn, urea, and alfalfa	68	66	80	53	76

[a] Calculated in the same manner as nitrogen free extract but given this title due to the possible presence of nitrogen compounds.

[b] Differences from control statistically significant at $P = < 0.05$.

and the remaining fiber probably was not subject to attack by bacterial enzymes in the ruminant's digestive tract. The digestibility of materials soluble in water, acids and bases, which would normally be called nitrogen-free extract, was also significantly reduced.

Similar trends were noted in Trial 2. The digestibility of dry matter nitrogen and of crude fiber was reduced when the furfural residues were included in the ration. In this trial ammoniated molasses provided a nitrogen digestibility only slightly better than that obtained with the ammoniated furfural products.

The digestibility of nitrogen from ammoniated DDMS in Trial 3 was strikingly lower than the coefficients obtained for urea nitrogen. The digestibility of dry matter from the ammoniated DDMS ration was also somewhat poorer than from other rations. The inclusion of 1 lb. of alfalfa hay in the ration increased the digestibility of all the nutrients studied.

Nitrogen balance data are included in Table 8. It may be seen that the amount of nitrogen apparently absorbed reflects the reduced digestibility of nitrogen from ammoniated products in all trials. The values from Trial 1 are somewhat higher than those in the two other trials, because nitrogen was overfed in this study, whereas it was fed at the minimum requirement in Trials 2 and 3. In Trial 1 the absorbed nitrogen from the ammoniated products was not as well retained as that from soybean oil meal. In Trial 2 residue No. 2 and ammoniated molasses provided absorbed nitrogen in a form which was retained to the same extent as nitrogen from soybean oil meal. Nitrogen absorbed from residue No. 1 was retained to a lesser degree. Absorbed nitrogen from ammoniated DDMS was

TABLE 8

Average nitrogen balance data obtained from three balance studies involving ammoniated products

Ration characteristics	Nitrogen			Per cent of	
	Consumed	Apparently absorbed	Retained	Absorbed *N* retained	Consumed *N* retained
	(g.)	*(g.)*	*(g.)*	*(%)*	*(%)*
		Trial I			
Residue A	133	68	24	34	18
Residue B	144	75	29	39	20
Residue C	133	63	22	36	16
Soybean oil meal	154	102	44	43	28
		Trial II			
Residue I	74	29	13	38	17
Residue II	78	33	17	49	22
Ammoniated molasses	79	35	16	46	21
Soybean oil meal	79	44	20	45	25
		Trial III			
Corn and urea	71	46	20	43	28
Corn and ammoniated DDMS	68	31	12	39	18
Corn, urea, and DDMS	71	46	26	56	37
Corn, urea, and alfalfa	77	51	23	45	30

retained poorly in comparison to that from other sources (Trial 3). The inclusion of DDMS in the ration apparently markedly improved the retention of absorbed nitrogen. The apparent improvement in the retention of digested nitrogen when DDMS was included in the ration is interesting in the light of in vitro experiments that have indicated increased cellulose digestion and utilization of nonprotein nitrogen by rumen bacteria when it was included in the media *(16)*.

DISCUSSION

The use and availability of nitrogen from ammoniated industrial by-products and ammoniated feeds by ruminants still require considerable investigation before a thorough knowledge will be obtained. It has been theorized that nitrogen from these sources might be as readily available to the microorganisms existing in the rumen as that from urea or natural protein. Several studies have indicated good or acceptable growth and use of nitrogen from these sources. A critical evaluation of some of these studies indicates that relatively low levels of nitrogen were supplied from the ammoniated products and that nitrogen from other sources was frequently fed at levels above the minimum requirements of the animals. Under these conditions one cannot obtain a critical evaluation of the utilization of nitrogen from any source.

The studies here reported indicate that ammoniated industrial by-products and some ammoniated feeds do provide a source of nitrogen which can be utilized to a certain degree by ruminant microorganisms. However, this nitrogen does not appear to be available to the same degree as nitrogen from natural protein and is not as well utilized as nitrogen from urea. Similar results have been reported by Ferguson and Reeves *(4)*.

Since the chemical combinations that occur when nitrogen is incorporated in feeds and industrial by-products are not well understood, it is not amiss to speculate that many of these compounds may be of a nature that is not subject to attack by enzymes normally occurring in the rumen. Unless this nitrogen is convertible to a utilizable form by dissolution or enzyme hydrolysis, one would not expect it to be available for use by the microorganisms.

Apparently, further research is necessary to find an ideal source of nonprotein nitrogen for ruminants. Ammoniated products manufactured under more subtle or better controlled conditions could result in an ideal source of a nonprotein nitrogen for microorganisms. This could be a product which would be attacked by some normal enzyme system occurring in the rumen and from which the nitrogen was liberated at approximately the same rate as the energy necessary for the synthesis of protein.

Another factor which enters into the feasibility of using ammoniated industrial by-products as a source of nitrogen for ruminants is the fact that many of them, as shown in this paper, contain relatively low levels of energy or available nutrients. It appears economically unfeasible to transport large amounts of relatively inert materials long distances for feeds. A source of nonprotein nitrogen which is concentrated or else contains appreciable amounts of energy would appear to be much more practical.

The possibility of toxic reactions must be considered in evaluating ammoniated products as a source of nitrogen. Recent reports by Richardson *et al. (14)* have indicated that animals fed fairly low levels of ammoniated molasses have shown nervous symptoms. Apparently, other workers have made similar observations. Until more is known about the chemical combinations appearing from products ammoniated under relatively severe conditions, caution should be used in the feeding of these materials.

SUMMARY

A series of studies on the in vitro and in vivo utilization of nitrogen from ammoniated industrial by-products and ammoniated feed stuffs has been reported. The results of both types of studies indicate that nitrogen from ammoniated products is not available to rumen bacteria or to the host to the same degree as is nitrogen from urea or from natural protein sources.

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ADDITIONS OF HAY TO CORN SILAGE TO MAXIMIZE FEED INTAKE AND MILK PRODUCTION[1]

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Early feeding trials have indicated that a stimulation of milk production resulted when part of the dry roughage was replaced by corn silage. Pratt and White *(7)* reviewed the early literature and found general agreement for increased FCM production per unit of dry matter with increasing amounts of silage in the ration of milking cows. Their own studies *(7, 12)* resulted in similar milk production and body weight changes for groups of cows fed approximately 18 and 36 lb. of corn silage per day in addition to hay and grain.

It is well accepted that silage as the only source of roughage will support rather high levels of milk production *(3, 6)*. With grass silage feeding, higher production has resulted from including various amounts of hay in addition to silage *(2, 5, 11)*.

This paper is a report of a series of feeding trials carried out to determine the amount of hay to be added to an ad libitum corn silage feeding regime which would result in maxima for dry matter intake and milk production.

EXPERIMENTAL PROCEDURE

Animals. Sixty cows were used in six trials carried out during the 1951-52 and 1952-53 barn feeding periods at three locations, viz., the Mountain Test Farm, Waynesville; the Coastal Plain Test Farm, Willard; and the Central Station, Raleigh, North Carolina. Since four treatments were to be compared, four cows at a time at a given location were selected for being similar in breed, age, stage of lactation, and live weight to make up a replicate. By breeds, the following number of replicates were used: Ayrshire 1, Guernsey 1, Holstein 2, and Jersey 11.

Rations and feeding procedure. The four cows in a replicate were simultaneously started on a 28-day standardizing period during which each cow was offered daily corn silage ad libitum, 1 lb. of hay per 100 lb. of live weight and 1 lb. of grain per 4 lb. of FCM *(4)*. In five of the six trials the hay fed was alfalfa with an estimated mean TDN content *(8, 9)* of 54 to 59% (moisture-free basis) and a protein content ranging from 13.6 to 17.3%. A 15.1% protein soybean hay with an estimated TDN content *(8, 9)* of 54% was fed to three replicates in the sixth trial. Among the trials the mean dry matter content of the corn silage

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varied from 19.8 to 25.0% and had an estimated TDN content of the dry matter *(8, 9)* of 64 to 67%. Conventional concentrate mixtures with a mean protein content of 14.3 to 22.2% were fed in these trials.

Immediately after the standardizing period, the four cows in each replicate were randomly assigned to receive one of four levels of hay continuously for a 56-day comparison period. The four levels of hay offered were 1.00, 0.50, 0.25, and 0.00 lb. per 100 lb. of live weight daily. All feeds fed were those described above. In the first half of the comparison period concentrate was fed at 1 lb. per 4 lb. of FCM produced in the last week of the standardizing period. For the second half the amount was reduced by 5%. The corn silage was fed essentially ad libitum, being provided at least three times daily.

Observations and analyses. The amounts of concentrate, hay and corn silage, fed and refused, were determined and recorded daily. All cows were milked twice daily; the weights were recorded, and aliquot samples were pooled for 1-week (in standardizing) or 2-week (in comparison) periods for fat test by the Babcock method *(1)*.

Cows were weighed on the first 3 days of the standardizing period and on the first and last 3 days of the comparison period. The 3-day averages were used for all calculations.

All feeds were analyzed by official methods *(1)* except for the determination of dry matter, for which 70° C. with vacuum was used.

An analysis of covariance was carried out on the values obtained for the comparison period with the corresponding values for the last 14 days of the standardizing period used as the covariable *(10)*.

RESULTS

Feed consumption. The mean dry matter intakes of grain, silage, and hay in the standardizing period and of grain in the comparison period were similar for the four treatment groups (Table 1). The intended levels of hay intake were not quite realized. The actual mean daily intakes for the groups in the standardizing period ranged from 0.81 to 0.86 lb. per 100 lb. of live weight. In the comparison period the intakes averaged 0.83, 0.47, 0.24, and 0.00 lb. of hay for the groups offered 1.00, 0.50, 0.25, and 0.00 lb. per 100 lb. of live weight, respectively. The decreased dry matter intakes which resulted when the lower levels of hay were fed can be readily seen in Table 1. These differences were very highly significant statistically ($P < 0.001$). This response of dry matter intake to level of hay in the diet was significantly quadratic ($P < 0.05$). This relationship was well described by the following equation:

$$Y = 21.38 + 10.40X - 6.60X^2$$

where Y equals the dry matter intake in pounds per cow per day and X equals the daily pounds of hay consumed per 100 lb. live weight in addition to silage, ad libitum. Using the above relationship, maximum dry matter intake was calcu-

TABLE 1

Mean daily dry matter intakes and milk and fat production for groups of cows offered the indicated level of hay to supplement corn silage

	Lb. hay per 100 lb. live weight				Standard error of a mean
	1.00	0.50	0.25	0.00	
	Mean daily lb. dry matter intake				
Standardizing period					
Grain	8.5	8.7	8.4	8.0	
Hay	6.9	6.8	7.0	6.9	
Silage	9.6	9.1	9.4	9.5	
Total	25.0	24.6	24.8	24.4	0.77
Comparison period					
Grain	7.6	8.0	7.4	7.5	
Hay	6.9	3.9	1.9	0.0	
Silage	11.2	13.2	13.5	14.1	
Total	25.7	25.1	22.8	21.6	0.67
Comparison total adjusted for standardizing total	25.5	25.1	22.7	21.7	0.46
	Mean daily lb. milk production				
Standardizing period	30.85	31.94	29.57	28.81	1.36
Comparison period	27.75	28.99	26.01	24.73	1.34
Comparison adjusted for standardizing	27.22	27.43	26.69	26.13	0.36
	Mean daily lb. fat production				
Standardizing period	1.425	1.513	1.394	1.431	0.064
Comparison period	1.303	1.389	1.248	1.246	0.060
Comparison adjusted for standardizing	1.316	1.327	1.288	1.255	0.024
	% fat				
Standardizing period	4.76	4.84	4.81	5.02	0.12
Comparison period	4.85	4.92	4.93	5.14	0.11
Comparison adjusted for standardizing	4.93	4.93	4.98	5.00	0.04
	Mean daily lb. FCM production				
Standardizing period	34.21	35.44	32.87	33.07	1.43
Comparison period	30.59	32.42	29.12	28.66	1.39
Comparison adjusted for standardizing	30.33	31.03	30.04	29.40	0.54

lated to occur at a level of 0.79 lb. of hay per 100 lb. live weight. The 95% confidence interval for this value was 0.42 to 1.16 lb., a range which brackets the highest level of hay fed in this study.

The dry matter intakes from silage (Table 1) are of interest. The cows remaining on the 1.00 lb. level of hay during the comparison increased their silage consumption only slightly (1.6 lb.), whereas the three groups receiving the lesser amounts of hay consumed appreciably more dry matter in the form of silage (4.1, 4.1, and 4.6 lb., respectively) during this period. Somewhat of a craving for dry roughage was observed in three of the 15 cows on the all-silage ration. This desire apparently diminished as the experiment progressed.

Milk production (Table 1). The same trend was observed for milk, fat, and FCM, with the lowest production in the zero hay group, increasing with level of hay to the 0.50 lb. group and dropping off again in the highest hay group.

Although these over-all treatment differences for FCM and pounds fat were not statistically significant, those for pounds milk approached significance ($P < 0.10$). The trend in FCM response to level of hay was essentially quadratic ($P \cong 0.10$) and was described by the following equation:

$$Y = 29.78 + 6.08X - 5.88X^2$$

where Y equals the FCM production in pounds per cow per day and X equals the pounds of hay consumed daily per 100 lb. live weight in addition to silage ad libitum. From this relationship, maximum FCM production was calculated to result at an intake of 0.52 lb. of hay per 100 lb. live weight. The 95% confidence interval for this value was 0.30 to 0.74 lb. of hay. No influence of the experimental rations on the per cent fat in the milk was found.

Live weight. The mean initial live weights for the four treatment groups arranged by decreasing level of hay were 886, 883, 864, and 859 lb., with a standard error of 21 lb. The mean live weight changes during the comparison for these groups in the same order as above were +23.5, +24.8, +23.4, and +21.8 lb., with a standard error of 5.4 lb. These weight changes were not significantly different.

DISCUSSION

These data confirm the belief that corn silage as the sole source of roughage will support rather high levels of milk production with moderate grain feeding. However, increases in dry matter intake and milk production can be realized by feeding hay in addition to the silage. As might be expected, the response of both criteria to increasing the level of supplemental hay is curvilinear.

The maximum for dry matter intake occurred at a higher level of hay feeding than did that for FCM production. The greater dry matter intakes at the higher levels of hay did not result in as wide differences in milk or FCM production as might have been anticipated. This response is compatible with early observations *(7)* that dry matter from silage is more valuable for milk production than that from hay.

Since the cows in this study were fed grain at 1 lb. to 4 lb. of FCM, it might be anticipated that wider differences in production would result from similar additions of hay at a lower level of concentrate feeding. This would be due, of course, to the necessity for the roughage to provide a greater portion of the total energy.

SUMMARY

Sixty cows were used in a series of feeding trials conducted at three North Carolina experimental farms over two winter-feeding periods to determine the optimum amount of hay to be used to supplement an ad libitum corn silage feeding regime for milk production. The four hay levels consumed were 0.83, 0.47, 0.24, and 0.00 lb. per 100 lb. of live weight. After a 28-day standardizing period, in which cows were offered silage ad libitum and 1 lb. of hay per 100 lb. of live weight, each cow was carried for a 56-day comparison period on one of the four experimental rations.

Dry matter intake was highest in the group which consumed 0.83 lb. of hay per 100 lb. of live weight with the calculated maximum intake being at 0.79 ± 0.37 lb. of hay. Milk, fat, and FCM production was highest for the group consuming the 0.47 lb. level of hay with the calculated maximum for FCM being at 0.52 ± 0.22 lb. of hay. Mean changes in live weight were essentially the same for the four treatment groups.

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AUTHORS' CORRECTIONS

"Lipase Activity in Fresh Milk as Related to Portions of Milk Drawn and Fat Globule Size," Vol. 38 (March, 1955), by W. R. Thomas, W. J. Harper, and I. A. Gould.

1. Page 316, Table 1, the column heading "Fat content" should read "Acid degree."

"On the Mechanism of Activation of Lipolysis and the Stability of Lipase Systems of Normal Milk," Vol. 38 (April, 1955), by N. P. Tarassuk and E. N. Frankel.

1. Page 438. Table 1, Experiment 2, line 3: "Foamed 2 minutes without antifoaming agent" should read "Foamed 12 minutes without antifoaming agent."

2. Page 439, line 3-b: "Lipase is a surface reaction" should read "Lipolysis is a surface reaction."

TECHNICAL NOTES

EFFECT OF FEEDING N, N′-DIPHENYL-PARA-PHENYLENEDIAMINE TO LACTATING DAIRY COWS ON CAROTENE UTILIZATION AND INCIDENCE OF OXIDIZED MILK FLAVOR[1]

N, N′-diphenyl-para-phenylenediamine (DPPD) has been used to retard storage losses of carotene in dehydrated alfalfa *(5)* and has been fed to poultry to increase storage of vitamin A *(10)* and carotenoid pigmentation *(9, 12)*. In addition, DPPD has exhibited antioxidant properties similar to those of vitamin E by markedly reducing the incidence of encephalomalacia when vitamin E low diets containing 2% fish oils were fed to chicks *(11)*. In light of these findings and of the possible role of vitamin E in the prevention of the oxidized milk flavor defect *(7)*, a study was initiated to test the effect of feeding DPPD to lactating dairy cows on their carotene utilization and on the incidence of the oxidized flavor defect in their milk.

Two experiments have been conducted to date. The first (I), conducted October, 1954, to April, 1955, consisted of four cows, two Guernseys and two Holsteins, chosen at random from animals recently calved in the University herd. These animals were fed a carotene-low ration[2] for a 13-week depletion period starting the 42nd day postpartum. After this depletion period, dehydrated alfalfa meal was added to the ration at the 15% level, which allowed a daily carotene intake of approximately 25.8 mg/100 lb. live weight. A 2-week stabilizing period followed, and then DPPD was added to the ration at a rate of 0.01% for two 3-week periods in a simple reversal trial *(8)*. A 3-week period, when no DPPD was fed, was allowed between the feeding periods to eliminate carry-over effects. On two consecutive days each 7-day period milk samples of the A.M. and P.M. milking were composited for determination of specific gravity, butterfat content *(2)*, and carotenoid and vitamin A content *(3)*. Once weekly blood samples were drawn for determination of carotenoid and vitamin A concentration *(6)*. Once weekly milk samples of the A.M. milking were obtained for organoleptic testing without and with the addition of 0.5 p.p.m. of Cu^{++}, as copper sulfate. These were tested in duplicate for the presence or absence of oxidized flavor independently by a panel of three judges 24 and 48 hours after milking.

[1] This study was made in part with funds provided by the Chas. M. Cox Company, Boston, Mass., and the Big-Y-Foundation, Norwich, Conn. The authors wish to thank Messrs. L. R. Dowd, A. C. Smith, and L. R. Glazier for their extensive assistance in conducting the organoleptic tests. Mr. R. E. Althaus, B. F. Goodrich Chemical Co., Cleveland, Ohio, kindly furnished the commercial grade DPPD.

[2] This ration consisted of 58% beet pulp, 35% dairy ration, 5% linseed oil meal o.p., 1% steamed bone meal and 1% iodized salt, was soaked with water, and was fed at a daily rate of 2.7 lb. per 100 lb. of live weight.

TABLE 1

Effect of feeding DPPD on plasma and milk carotenoids and vitamin A

Criterion	Expt.	Ration		Standard deviation of an observation	L.S.D. @ P = 0.05
		No DPPD	With DPPD		
Blood plasma					
Carotenoids *(γ%)*	I	390	403	19	57
	II	955	1064	114	140
Vitamin A *(γ%)*	I	28.9	29.5	2.1	6.4
	II	37.4	42.2[a]	2.9	3.6
Milk carotenoids					
Concentration *(γ%)*	I	17	16	4	13
	II	22	22	5	7
Per gram of fat *(γ)*	I	3.4	3.6	0.2	0.8
	II	5.2	5.0	0.9	1.2
Total daily secretion *(γ)*	I	1780	1683	160	486
	II	4571	4865	1017	1413
Milk vitamin A					
Concentration *(γ%)*	I	12.6	12.1	0.4	1.1
	II	17.9	20.9	5.0	7.0
Per gram of fat *(γ)*	I	3.3	3.6	1.1	3.2
	II	4.1	4.7	1.0	1.4
Total daily secretion *(γ)*	I	1567	1580	229	697
	II	3617	4509	1070	1486

[a] $P < 0.05$.

TABLE 2

Effect of feeding DPPD on milk production, per cent fat, and per cent samples free from oxidized flavor

Criterion	Expt.	Ration		Standard deviation of an observation	L.S.D. @ P = 0.05
		No DPPD	With DPPD		
Milk production					
Yield *(lb.)*	I	28.6	29.0	2.5	7.6
	II	46.2	47.8	3.5	4.2
Fat *(%)*	I	4.1	4.0	0.3	1.0
	II	4.1	4.2	0.2	0.2
Per cent samples free from oxidized flavor					
Raw milk					
24 hours *(%)*	I	85.4	95.8	12.2	37.0
	II	96.1	100.0	5.0	6.1
48 hours *(%)*	I	79.2	91.7	31.7	96.5
	II	93.0	100.0	9.0	11.0
Raw milk plus 0.5 p.p.m. Cu^{++}					
24 hours *(%)*	I	41.7	100.0	30.3	92.3
	II	13.3	100.0[a]	23.6	28.9
48 hours *(%)*	I	37.5	93.8	31.9	97.0
	II	10.9	100.0[a]	21.9	26.8

[a] $P < 0.001$.

The second experiment (II), conducted from February to April, 1955, consisted of eight Holstein cows averaging 114 ± 20 days in lactation and selected for producing milk which would develop oxidized flavor within 24 hours after the addition of 0.5 p.p.m. of Cu^{++}. Each animal was fed approximately 65 lb. grass silage, 10 lb. alfalfa hay, 8 lb. 16% dairy ration, and 4 lb. citrus pulp. Two grams of DPPD was fed daily per cow during two 15-day periods in a simple reversal trial. For 10 days between the two feeding periods no DPPD was fed so that all animals again would produce susceptible milk. Once during the latter part of each feeding period milk and blood samples were taken for analyses as in Experiment I. Milk samples of the A.M. milking were taken every second day for organoleptic testing, as before, by two judges.

From the data in Table 1 it appeared that, in general, the feeding of DPPD had a stimulatory effect on the utilization of carotene as exhibited by higher carotenoid and vitamin A values of the blood plasma and the milk. The fact that the magnitude of the differences was greater for Experiment II than for Experiment I may possibly be due to less efficient utilization of carotene by cows on the atypic ration used in the first experiment *(4)*.

The data in Table 2 showed that there was a very definite reduction in the occurrence of oxidized milk flavor in the DPPD-supplemented group. In comparing the results of the two experiments it should be noted that the cows in Experiment I were not selected for producing milk susceptible to copper-induced oxidized flavor, and therefore the results were more variable and not as pronounced as in Experiment II.

None of the milk samples contained off-flavors which could be directly attributed to the feeding of DPPD. Twice during each feeding period in the second experiment individual pasteurized milk samples were tested for inhibitory action against an active commercial cheese culture by a resazurin reduction test *(1)*, and no differences in reduction time were observed between ration groups.

No adverse effects on the cows' health were observed or feed refusals encountered attributable to the feeding of DPPD.

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THE MECHANISM OF ACTIVATION OF LIPOLYSIS BY FOAMING

In the April issue of this Journal, Tarassuk and Frankel presented evidence that foaming rather than agitation is the key to the activation of lipolysis in pipeline milkers. That is, of course, the obvious interpretation of our experiments. A gentle treatment which produces foam in warm raw milk is much more hazardous than violent agitation without foam production.

Tarassuk and Frankel offer an interesting hypothesis to explain why this is so, but they have overlooked an important fact which needs explanation. Mild treatment which produces foaming causes a great increase in the number of very small fat globules. Violent agitation in a Waring Blendor completely filled with milk does not do this. This is the fact that we have found difficult to explain. Why does foaming produce a mild degree of homogenization? The work of Nikolous King is undoubtedly pertinent to this problem, but it is still difficult to account for all of the facts.

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EFFECT OF FEEDING ANTIOXIDANT (CACAO SHELL) ON THE STABILITY OF THE MILK FAT[1]

It is logical to assume that for an antioxidant to exert its maximum effect on milk fat it should be applied as soon as possible. The earliest time would be to add the antioxidant to the feed of the cow. This would be effective only if the antioxidant was transmitted by the cow into the milk from the feed.

Cacao shell was selected as the test material in this study because it contains antioxidative substances for milk fat *(8)*, is relatively inexpensive, and is nontoxic when fed in limited amounts to animals *(1, 6)*. A Jersey cow whose milk was susceptible to the development of an oxidized flavor was used for the feeding tests.

The usual basal grain ration fed to the University herd was supplemented by 2.5% of ground cacao shell from roasted fermented beans. The total feed ration consisted of 9.23 lb. of grain-cacao shell mixture, approximately 20 lb. of chopped green corn, and a few pounds of alfalfa hay. The cow was kept on pasture except when she was fed the concentrates and was milked twice daily. The daily cacao shell intake was 0.23 lb., which was the maximum amount that the cow would consume.

[1] This article is based upon part of a Master of Science thesis submitted to the Graduate School by Kazys Blazys, October, 1953. Contribution No. 999 from the Mass. Agr. Expt. Sta.

The feeding tests carried out during the months of August and September, 1952, were divided into four periods: *(a)* pre-experimental feeding, *(b)* 1 week experimental feeding of cacao shell, *(c)* 2 weeks post-experimental feeding, and *(d)* 4 weeks post-experimental feeding.

The antioxidative effect of feeding cacao shell was determined by the thiobarbituric acid (T.B.A.) test *(2)* and taste tests on the milk; and by T.B.A. *(9)*, peroxide value, color, and taste tests on the butteroil. At the end of each feeding period, three successive milkings were combined to obtain the required amount of milk and butteroil for performing the tests. After each milking, the milk was cooled rapidly to 50° F. To a portion of the collected sample, 4 p.p.m. of copper ($CuSO_4$) was added to hasten oxidative deterioration. It was then stored in a refrigerator at 50° F. The milk samples were tested for flavor and submitted to the T.B.A. test immediately and after 2, 3, and 4 days of storage.

Cream was separated from the remaining portion of the collected milk and churned into butteroil, which was prepared according to the method of El-Rafey *et al. (3)*. The butteroil was stored in amber bottles at 50° F. The stability of butteroil to which copper was added was determined by the Swift Fat Stability Test *(10)*.

TABLE 1

Antioxygenic effect of cacao shell fed to the cow on stability of milk[a]

Milk stored at 50° F.	Pre-experimental feeding		After feeding cacao shell for 1 week		After 2 weeks post-experimental feeding of cacao shell		After 4 weeks post-experimental feeding of cacao shell	
	T.B.A. test optical density	Oxidized taste[b]	T.B.A. test optical density	Oxidized taste[b]	T.B.A. test optical density	Oxidized taste[b]	T.B.A. test optical density	Oxidized taste[b]
Fresh milk	0.095	−	0.101	−	0.098	−	0.109	−
After 2 days	0.146	+	0.138	−	0.134	−	0.143	+
After 3 days	0.178	++	0.168	+	0.159	+	0.170	+
After 4 days	0.208	++	0.198	++	0.185	++	0.203	++

[a] Four p.p.m. of copper was added to all samples.
[b] Degrees of oxidized taste indicated as: − absent, + slight, and ++ definite.

Results

The antioxygenic effect on the milk, when 0.23 lb. of cacao shell was consumed daily for 1 week, is indicated in Table 1. The cacao shell increased the resistance of the milk to oxidative deterioration as evidenced by taste and T.B.A. tests. In the T.B.A. test, a decrease in optical density indicates a decrease in oxidative spoilage. The maximum antioxidative effect of the cacao shell, as measured by the T.B.A. test of milk, was found at the end of the 2 weeks post-experimental feeding period. This result is in accord with reports by Frye *(4)*, Hilditch *(5)*, and Krukovsky *(7)* that the maximum effect of feed on the changes in milk fat composition and its resistance to oxidative deterioration may be reached in approximately 2 to 3 weeks after change in feed.

The antioxygenic effect on butteroil obtained from the milk when 0.23 lb. of cacao shell was consumed daily for 1 week was most pronounced at the end of the 2 weeks post-experimental feeding period. This result was observed for all tests made with butteroil; however, not all results are presented in Figure 1 because of space limitation.

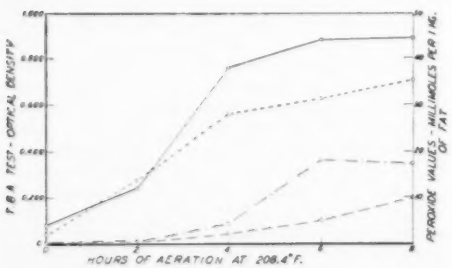

FIG. 1. Antioxygenic effect of cacao shell fed to the cow on the stability of butteroil containing 4 p.p.m. of added copper.

——— Pre-experimental feeding (T.B.A. test)
– – – – – Two weeks post-experimental feeding (T.B.A. test)
——— Pre-experimental feeding (peroxide values)
– – – – – Two weeks post-experimental feeding (peroxide values)

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PEOPLE and EVENTS
in the Dairy Science World

Chicago Dairy Industry Leader Honored

In recognition of his contributions to human welfare of the state and nation, P. D. SANG, vice-president of the Goldenrod Ice Cream Co., Chicago, was honored by being granted a degree of Doctor of Humane Letters at the 90th annual commencement of Lincoln College, Lincoln, Ill., on May 29. Mr. Sang, a firm believer in higher education and research, has traveled extensively abroad to study social and economic conditions. He is active in social welfare work and participates in city, state, and international trade association work. Mr. Sang operates one of the most efficient ice cream plants in the country and, in addition, he has found time to study American history as a hobby and is a collector of rare Americana volumes and manuscripts. The dairy industry can be proud of Dr. Sang's achievement and the honor that has been bestowed upon him.

When our busy industrial leaders take the time necessary to establish a record of unselfish service to society comparable to that gained by Dr. Sang, free enterprise need not fear for its future in America. It is also a healthful sign when centers of learning and culture recognize worth-while contributions made by industrial leaders outside the usual channels of commerce.

Honorary Degree Conferred Upon A.D.S.A. Member

MRS. ETHEL A. MARTIN received the Honorary Degree of Doctor of Science from South Dakota State College at commencement exercises held May 23. Mrs. Martin served as director of the Dept. of Nutrition Services of the National Dairy Council for 22 years, retiring in 1951. Since that time she has remained active in the field of nutrition and has served the Council as special consultant.

Dr. Martin's career has included many honors. In 1949 she was selected by the U. S. Dept. of State as a delegate to the 12th Intern. Dairy Congress held in Stockholm—the only woman to serve in that capacity. She is the author of many professional articles and books and is a member of the USDA's Advisory Committee on Food and Nutrition.

During earlier years, Mrs. Martin taught home economics at Texas State College for Women, the Univ. of Illinois, and the Univ. of Chicago. She is an active member of the American Dietetic Assoc., American Home Economics Assoc., A.A.A.S., and A.D.S.A.

Dairy Scientist Wins Lilly Award

W. A. WOOD, dairy bacteriologist in the Univ. of Illinois Dept. of Dairy Science, was awarded the coveted Eli Lilly prize in New York City May 11 in recognition of his outstanding contributions to the fundamentals of bacteriology and immunology.

The $1,000 prize and citation scroll were presented by the Society of American Bacteriologists as the highlight of the annual meeting. The award is made annually to a young scientist under 35 years of age for outstanding achievements in research in bacteriology. Dr. Wood was cited for fundamental research in the fields of amino acid and carbohydrate metabolism and the mechanisms of vitamin synthesis by bacteria. He graduated from Cornell Univ. in 1947 and received his Ph.D. from Indiana Univ. in 1950. He joined the staff at Illinois in February, 1950.

Parfitt Recognized for 10 Years Activity in Dairy Equipment Standards

In recognition of his 10 years service as chairman of the Sanitary Standards Subcommittee of Dairy Industry Committee, in which period he has also presided at the joint meetings of the 3-A Sanitary Standards Committees, E. H. PARFITT, executive secretary of the Evaporated Milk Assoc., was presented with a gold wrist watch.

In making the award for the joint group, T. A. BURRESS, The Heil Co., a director of D.I.S.A., observed that Dr. Parfitt had exerted a steadying and constructive influence on the deliberations of the standards conferees. He said that from among original wide divergences of opinion involving equipment makers, equipment users, and sanitarians there had emerged under Dr. Parfitt's guidance solid basic 3-A Sanitary Standards concurred in by all interested segments of the industry and meriting the country-wide approval of regulatory men.

Tennessee Features College Course in Public Relations

Realizing the importance of public relations in the training of students, C. E. Wylie, head of the Dairy Husbandry Dept. at Tennessee, has instigated a course covering this subject and designed primarily for dairy majors. This was brought out in a recent issue of the Pet Milk Co. magazine in a feature story about the workings of the Dept. at Tennessee in which it is stated: ". . . since public relations constitutes a large part of the work of fieldmen and

often plant managers, it is common sense that dairy majors should be trained in the basic principles of getting along with people."

O.S.U. Holds Dairy Tech Career Day

The second annual Dairy Technology Career Day was held on the Ohio State campus April 16. Eighty-six high school seniors visited the campus as guests of the University and the dairy industry. The visitors toured the Univ. dairy plant and the dairy building to view exhibits illustrating various phases of dairy technology. "The Miracle of Milk," a movie depicting dairy product processing, was shown, as well as a movie entitled "The University Story," which described various phases of student life on the campus. I. A. GOULD, departmental chairman, presided over a brief formal program, which acquainted the students with the opportunities in dairy technology. After a luncheon at the Ohio Union, industry leaders spoke briefly on the future of the dairy industry and indicated what a young man can expect in this field. Students majoring in dairy technology assisted the faculty as hosts.

This all-expense paid visit to the campus was sponsored jointly by the Univ., the Ohio Dairy Products Assoc., the Dairy Technol. Societies, and local dairy organizations. This is part of an over-all program in the state to secure more students for the field of dairy technology.

Sutton Goes to India

T. S. SUTTON, Asst. Dean of the College of Agriculture, The Ohio State Univ., and former editor of the *Journal of Dairy Science*, is now on a 40-day trip to India to make a preliminary study of educational and research needs. In September he expects to return to India for a 2-year period as head of an educational mission, the primary purpose of which is to set up a college program patterned after our land grant college plan. This will include teaching, research, and extension in a coordinated program.

First Dairy Technology Society in U.S.A. Celebrates Silver Anniversary

The Chicago Dairy Technol. Soc. celebrated its 25th birthday, May 10. The meeting was attended by members and their wives. Approximately 550 were present for the dinner and entertainment. Of the 23 past presidents 19 are living; 11 of these were present and received past president pins from W. H. ANDERSON, the present leader of the society.

The society honored P. H. TRACY, who has served as secretary of the group since its founding in 1930. He was presented with a gold plated life membership card, a distinguished service plaque, and a set of matched luggage. C. F. WEINREICH, the second president of the society assisted with the presentations. The Chicago group was the first dairy technology society of its kind to be organized in this country.

Committee Praised for Work on 3-A Dairy Equipment Standards

"Each of you can take justifiable pride, I believe, in the progress you have made together toward assuring that milk—perhaps the one food most necessary for proper health and growth—and the products derived from milk, are both as wholesome as modern technology can make them and as accessible to all as possible." Thus spoke BRADSHAW MINTENER, Asst. U. S. Secretary of Health, Education, and Welfare, before the semi-annual joint meeting of 3-A Sanitary Standards Committees, April 27, at Kenwood Country Club, Bethesda, Md.

These committees include representatives from the Intern. Assoc. of Milk and Food Sanitarians, U. S. Public Health Service, and the Dairy Industry Committee. Mr. Mintener reviewed the work of the Dept. of Health, Education, and Welfare in fields related to milk and milk products, its participation in such programs as the Interstate Milk Shippers Certification Program, and its cooperation with American Butter Inst. in a general cream improvement program and related activities of the Food and Drug Administration. He paid tribute to Dairy Industries Supply Assoc. and Milk Industry Fdn. for assisting the U. S. Public Health Service with equipment and technical counsel in studies relating to Q-fever. He mentioned recent research in the application of atomic radiation to food processes involving milk and dairy products.

Earl Weaver Takes Assignment in South America

EARL WEAVER, since 1937 head of the Dairy Dept. of Michigan State College, will leave around July 1 to head an educational commission in Colombia, S. A. He will be gone 2 years. N. P. RALSTON of the Dairy Production Division has been appointed head of the department. The two colleges of Colombia are being sponsored by the Michigan State College administration. M.S.C. has provided a portion of the faculty for several years. It is believed that this policy has become so important and holds such promise for the future of international relations that it is the desire of the M.S.C. administration to send men in the higher echelons to further develop this work. Because dairying is regarded as one of the great needs of that country, Dr. Weaver has been prevailed upon to accept the position.

First John Brandt Fellowship Awarded at Minnesota

W. D. SCHULTZE will receive the first John Brandt Memorial Fellowship for graduate study in dairy industry at the Univ. of Minnesota. Announcement of the award was made by J. B. FITCH, head of the Dairy Husbandry Dept.,

and CARL LEAF, Litchfield, Minn., president of the John Brandt Memorial Foundation. The fellowship has a value of $1,500.

A native of Philadelphia, Schultze is completing work on a Ph.D. degree. His thesis research deals with the characteristics of certain bacteria that have a direct influence on the quality of milk and other dairy products. Schultze was granted the B.S. degree from the Univ. of Pennsylvania in 1948 and received an M.S. degree in bacteriology from the Univ. of Wisconsin in 1951. He enrolled in the Graduate School of the Univ. of Minnesota in the fall of 1952.

The John Brandt Memorial Foundation was set up by friends of the late John Brandt, widely-known Minnesota dairy industry leader, who died in 1953. Income from the Foundation's trust fund is to be used to foster research to improve the quality of dairy products, to expand their use, and to develop new products. It is planned to give one or two $1,500 fellowships each year to promising students in the dairy sciences.

Illinois Holds Career Day

The division of Dairy Technology at the Univ. of Illinois held its first Dairy Technology Career Day April 15. Over 250 students, members of industry, and faculty attended the luncheon. The day was spent in seeing the various points of interest on the campus, the final event being a visit to the dairy manufactures building, where the guests were given a chance to view the plant in full operation.

At the luncheon P. H. TRACY announced the availability to qualified high school seniors of 22 $1,000 scholarships given by the Illinois dairy industry for study of dairy technology at the University of Illinois. The 140 high school students present for the event were sponsored by dairy companies throughout the state.

News from Montana State College

At his request, J. A. NELSON has been relieved of his executive duties as dean of the Graduate Division. He will devote full time to teaching and research in the Dairy Industry Dept., of which he has been head for the past 26 years. Rapid expansion of both graduate work and the dairy industry activities made this change inevitable. LEON JOHNSON, professor of chemistry and executive director of the Research Foundation, will succeed Nelson as graduate dean on July 1, 1955.

E. A. KEYES has been added to the list of approved Jersey cattle judges by the board of directors of the American Jersey Cattle Club. Dr. Keyes, a member of the Montana State College dairy industry staff, has had many years of experience in judging dairy cattle at fairs and cattle shows. He served as teacher and research worker at the Univ. of Vermont and at Pennsylvania State Univ. before joining the Montana staff 10 years ago.

The 20th annual Dairy Industry Short Course will be held at Montana State College Nov. 14-16, 1955. The instruction will be designed to meet the present day problems of the dairy industry. A dairy products contest will be held in connection with the course.

Negotiations are under way for the purchase of a 160-acre farm one mile from the campus to be used as dairy production research and instruction center. The present dairy production set-up adjacent to the campus is to be abandoned and all the dairy production activities moved to the new location. The latest methods and future trends in milk production will be considered in equipping the new dairy farm.

Kentucky Events

H. B. MORRISON, professor of dairying at the Univ. of Kentucky, has been appointed dairy food processing advisor with the Foreign Operations Administration. Dr. Morrison will leave June 22 for a period of orientation in Washington, D. C., after which he and Mrs. Morrison will fly to Cochabamba, Bolivia. For 2 years he will supervise development and training of personnel for a dairy plant that will bottle milk and make butter, cheese, and dry milk.

W. F. LEWALLEN has been appointed research assistant in dairy technology at the Univ. of Kentucky. He received his B.S. degree from the Univ. of Tennessee in 1953.

S. A. PORTER was named honor guest at the annual Kentucky Dairy Club honor banquet held April 27. The award was presented to Mr. Porter for his outstanding contributions to dairying in his area while serving as county agent in Campbell County for over 25 years. Another feature of the banquet was the awarding of prizes to winners in the student dairy cattle and dairy products judging contests.

Eighty-two senior high school boys attended U. K. Dairy Career Day on April 27. This event was conducted by the Dairy Section. These students were recruited and sponsored by 19 dairy organizations and friends of the industry. After a tour of the campus the students were entertained at luncheon. They were shown the University's dairy facilities and were guests at the Dairy Club's annual honors banquet.

Frozen Semen Conference at Maine

A Frozen Semen Conference was held on the Univ. of Maine campus April 19 and 20. The meeting was opened by G. F. Dow, associate

director of the Maine Agr. Expt. Station, who welcomed the delegates. H. H. BRUGMAN, assistant professor of animal husbandry, opened the discussion period with an explanation of new equipment that has been designed and built at the Maine Station. Visiting speakers during the 2-day conference were J. W. BARTLETT, Rutgers Univ., R. W. BRATTON, Cornell Univ., RALPH CORBETT, Univ. of Maine, J. O. ALMQUIST, Pennsylvania State Univ., and PAUL HELLER, Connecticut Artificial Breeding Coop. Fifty-seven delegates from 18 states and Canada attended the conference. Transcripts are available at cost.

Food and Container Institute Publishes Important Report

M. S. PETERSON, chief, Scientific Publications and Reports office of the Quartermaster Food and Container Inst. for the Armed Forces, 1819 W. Pershing Road, Chicago, has announced the availability of copies of the proceedings of a symposium entitled "Methods for Evaluation of Nutritional Adequacy and Status." This 313-page book covers such subjects as the evaluation of protein, vitamin, and mineral adequacies, and the evaluation of nutritional status of populations. The book would be a valuable addition to the nutrition library.

Montgomery Ward Decision and Co-ops

The recent Wolfson vs. Avery (Montgomery Ward) case decided by the Illinois Supreme Court held that the Illinois Constitution prevents staggered terms for corporation directors, according to NORMAN KRAUSZ, farm law specialist of the Univ. of Illinois College of Agriculture. The reason the court gave for this decision is that staggered elections reduce the effect of the stockholder's vote. Only by allowing shareholders to cumulate their votes at an election of the entire board at one time can a shareholder get the maximum effect from his voting power.

This point can be illustrated best with figures. Assuming that 200 shares are to be voted, here's the situation:

Three directors up for election
51 shares are needed to elect one director

Nine directors up for election
21 shares are needed to elect one director

Staggering elections to elect only part of the board each year means that it takes a much larger number of shares to put a director into office.

The decision declaring staggered voting illegal would seem to apply to farm co-ops in the same way as it applied to business corporations, Krausz states. In practice, both corporations and co-ops allow staggering in the terms of directors. If this staggering provision in the corporation act is unconstitutional because it reduces voting power, then the staggering system used by the co-ops should be unconstitutional for the same reason.

Thus, it may become necessary for co-ops, if challenged by a shareholder, to drop the staggered voting system now widely used and to adopt an "elect all directors at once" system. Nonstock co-ops are seemingly not affected. The constitutional right to cumulate votes applies to shareholders, and there are none in these nonstock co-ops.

The Montgomery Ward decision does not mean that all directors presently in office hold office illegally; they are at least "de facto" directors and as such can continue to carry on the affairs of the organization—until the next election.

Interstate Shipment of Imitation Ice Cream Approved

Imitation ice cream or frozen dessert containing vegetable fat can be shipped in interstate commerce, according to the Food and Drug Administration, if the packages are conspicuously labeled "imitation ice cream" in letters of uniform size and prominence.

Completed Theses

Ph.D. Degree:

R. S. ADAMS—Some physiological effects of feeding filled milks to dairy calves. Univ. of Minnesota.

C. B. HARTLEY—A study of the cultural and physiological characteristics of an organism isolated from the intestinal tract of a calf. Univ. of Minnesota.

STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

Florida Dairy Club Holds Bar-B-Q

Annually the Dairy Science Club sponsors an event for the Dairy Science Dept. staff, workers, and extension men and their families. This year the event was held in the form of a beef Bar-B-Q. Afterward, a musical program was enjoyed by the more than 100 persons present.

Texas A. & M. Honors Mendes France

The Dairy Club at Texas A. & M. is known as the "Kream and Kow Klub." Its primary objective is to raise money to support various judging teams along with maintaining an interest in the dairy field. This year a prominent breeder donated a Jersey heifer to the club. This heifer will be raised, trained, fitted, and shown in the Austin Livestock Show, then sold in September.

Each year the club presents two prominent dairymen in the state with honorary memberships. In addition, this year P. MENDES FRANCE was presented with an honorary membership in the form of a special certificate.

Texas Tech Chapter Events

CHARLES WALES, a member of the Texas Tech Dairy Industry Club, was presented with a gold key signifying All-College honors at a recognition service on March 13. He had been recognized for his outstanding scholarship activities on three previous occasions.

Senior students made a 9-day tour of dairy processing plants in April. In addition, visits were made to paper and glass bottle plants and a sugar refinery.

The 17th annual Club banquet was held April 22. The judging team was honored, and the William's Memorial Scholarship was awarded to the outstanding junior student.

W.S.C. Club Activities

The Washington State College Dairy Club has two money raising projects. The production students currently have four heifer calves which will be fed for a short time, shown at the "Little International," and then sold at auction. The manufacturing students have installed an ice cream bar vending machine in a dormitory. This machine is so popular it must be serviced daily. A milk vending machine will be installed soon. Money made on these projects is used to support judging teams.

Annually the dairy club sponsors a banquet for those attending the Dairy Institute. Members of the club plan all the details, such as arrangements for the hall, menu, decorations, and program. The president of the club acts as toastmaster.

This chapter took part in the 43rd Little International on April 16. Over 125 students majoring in animal, dairy, and poultry husbandry competed for fitting and showmanship awards. The grand champion fitter was SHIRLY HENRICKSON and the grand champion showman was CHARLES BARTH.

Penn State Chapter Holds Annual Dairy Exposition

The annual Dairy Exposition at Penn State College was held May 17. Main features of this event included dairy cattle and dairy products judging contests, cattle fitting and showmanship competition, an ice cream mold-making contest, the selection of a dairy queen, and various displays in the dairy buildings. An added attraction this year was a milking contest for the Ag Faculty. The event was climaxed with an awards banquet.

O.S.U. Dairy Tech Students Form Council

The need for maintaining a high level of morals and enthusiasm among undergraduate students was responsible for the formation of a council by students majoring in dairy technology at the Ohio State Univ. Prime objectives include coordination of student activities and the improvement of student-faculty relations. The council is composed of three members from each class and will serve for a period of three quarters.

O.S.U. Dairy Science Students Hold Honor Banquet

The dairy science students at O.S.U. held their annual honor banquet April 22. This banquet, initiated in 1951, honors two men, one living and one deceased, who have made outstanding contributions in the field of dairy production. Those honored this year were I. D. HADLEY, secretary of the Ohio Holstein Assoc., and S. M. SALISBURY, Dairy Science Dept., who passed away on November 26. Enlarged photographs were presented to the University and will be hung in the Dairy Science Hall of Fame.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

The Rise and Fall of State Control of Consumer Milk Prices

R. W. Bartlett

Department of Agricultural Economics, University of Illinois, Urbana

In reviewing the current dairy situation one finds himself face to face with four important facts:

1. In 1954 the federal government purchased 320,000,000 lb. of butter, 277,000,000 lb. of cheese, and 645,000,000 lb. of nonfat solids. The milk equivalent of the butter and cheese purchased was 9 billion lb., or 7.3% of total milk production. This volume of milk was in excess of that sold through regular commercial channels.

2. Nutritionists recommend a minimum daily consumption of 1 qt. of milk for children and 1 or 1½ pt. for adults. Actual consumption falls far below these amounts.

3. People, particularly those in the medium- and low-income groups, drink more milk when milk prices are low than when they are high. This fact has been established by many scientific studies.[1] (Figure 1).

4. State control of consumer prices of milk tends to result in higher prices than prevail when these prices are arrived at on a competitive basis.[2]

When these facts are added together, it is evident that insofar as the cities included in recent studies were representative of all cities under state control and under competition, state control of consumer prices constitutes a legalized monopoly which is definitely against the public interest.

To What Extent Do State Governments Still Control Consumer Prices of Milk?

Primarily as a result of the depression between 1933 and 1940, 26 states and the federal government enacted legislation to fix prices which consumers should pay for milk. Together these states have a population of 100,600,000, or two-thirds of the total population. By the end of 1940, state control laws had been repealed or allowed to lapse in eight states—Delaware, Maryland, Michigan, Ohio, South Dakota, Texas, Utah, and Washington—and, after only a few months of operation, fixing of consumer prices by the federal government was abandoned on January 1, 1934.

Since 1941, seven other states—Connecticut, Indiana, Massachusetts, New Jersey, New York, Oregon, and Wisconsin—discontinued state control of consumer milk prices. In Oregon, state control of consumer milk prices was discontinued in November, 1954, as a result of a referendum by voters of the state. Ending of state control was accompanied by a 2-cent reduction in consumer prices in the Portland area and several other Oregon markets.

At present (May, 1955) 12 states—Alabama, California, Florida, Georgia, Montana, New Hampshire, Maine, Pennsylvania, Rhode Island, Tennessee, Vermont, and Virginia—have laws that establish minimum prices at which milk can be sold to consumers. In 1950 these states had a population of 39,200,000, or 26% of the total population. If Pennsylvania and California eliminated state price control, a situation which seems likely within the next 2 years, this would leave only 19,100,000 people under this type of control, or one-eighth of the total population.

What About Fixing of Consumer Prices?

The path of governmental fixing of consumer prices has been strewn with trials and tribulations. The federal government abandoned fixing of retail prices within a few months after it was initiated, primarily because its regulations could not be enforced. Difficulties accompanying the enforcement of consumer prices were also an underlying reason for abolishing state price control in several of the states which had enacted price legislation.

When a state legislature authorizes a board or commission to fix consumer prices, it is granting a monopoly control to this agency, supposedly in the interest of the public. As one studies the long-drawn-out and costly hear-

[1] *Illinois Farm Economics*, March, 1954, p. 1510, Footnote 1.

[2] Ibid. pp. 1509 to 1512.

ings dealing with consumer milk prices and then studies the decisions made, presumably based upon the evidence presented in these hearings, he becomes somewhat skeptical as to whether the decisions made are in line with public interest. This skepticism enlarges into a reality as one reviews the results of recent studies dealing with this problem.

An analysis of consumer price changes in 50 cities from 1929 to November, 1953, when subdivided into three groups showed (See Note 2):

1. In November, 1953, the lowest reported store price in 17 competitive markets averaged 20.1 cents per quart, or 3.1 cents less than the single-quart, home-delivered price (23.2 cents). In ten of the 17 markets, the store price was three cents a quart, or more, below the home-delivered price.

2. In sharp contrast, in November, 1953, the lowest store price in 18 state-controlled markets averaged 23.6 cents per quart, or only 0.5 of a cent per quart less than the single-quart, home-delivered price (24.1 cents). Ten of the 18 markets had no store differential.

3. The difference between home-delivered prices and store prices in the 17 competitive markets has been increasing in the past 24 years. In 1929, the difference between these two prices averaged only 1 cent per quart. By November, 1953, this difference had increased to 3.1 cents per quart.

4. In sharp contrast, in the 18 state-controlled markets the average difference between the single-quart, home-delivered price and the lowest store price was the same in 1929 as in November, 1953—only 0.5 of a cent per quart.

5. The lowest reported store price in the 18 state-controlled markets in November, 1953, averaged 23.6 cents per quart, or 3.5 cents per quart higher than the store price (20.1 cents) in the 17 competitive markets.

What Effect Did Low Store Prices Have on Sales of Milk?

This study in 50 cities showed two important facts: *(a)* that from November, 1952, to November, 1953, store prices in Cleveland and Columbus (Ohio), and Detroit, Michigan, were reduced more than in any of the other cities and *(b)* that milk sales in these markets increased faster in 1953 than in any of the other cities of comparable size for which sales data were available.

Among markets of one million population, or over, the 1953 sales of milk in Cleveland were 7.1% higher than in 1952; in Detroit, 5.9% higher; and in ten cities of this size, the 1953 sales averaged 1.8% higher than in 1952. Among cities of less than a million population, the 1953 sales of milk in Columbus were 11.1% higher than in 1952; in Wichita, Kansas, 10.2% higher, and in 20 federal-order cities, 6.1% higher.

No distributor in a state-controlled city could legally reduce consumer milk prices without sanction of the governing agency. Hence, few if any state-controlled markets have store prices as low as those reported for Chicago, Cleveland, Columbus, Detroit, Kansas City, and many other cities not under state control.

Store prices in Cleveland and Columbus decreased more in 1953 than those in any of the other cities. In November, 1953, consumers in both Cleveland and Columbus could buy milk for 16 cents a quart in quantity lots. These prices were 5 cents a quart less than the home-delivered prices in single quarts (21 cents). Detroit had a store price of 17.5 cents a quart in November, 1953, or 4.5 cents less than the home-delivered price (22 cents).

What Effect Did Increased Milk Sales Have on Dairymen's Income?

Increases in milk sales help farmers as well as consumers. Higher sales in Cleveland in 1953 gave dairy farmers $550,000 more income than they would have received had this milk been sold for manufacturing uses. Increases in dairymen's income have also resulted from higher sales in Columbus, Detroit, and other federal-order markets where consumer prices have been competitive.

Does State Price-Fixing Curb the Use of Inventions?

When originally set up most of the milk control boards in the United States fixed the price of milk sold in paper containers at 1 cent a quart higher than that for milk sold in glass bottles. This practice was based on cost comparisons which showed that distribution costs in paper containers were higher than costs in glass bottles. (More recent studies have shown distribution costs in paper containers to be lower.)

Looking at this practice in perspective one can logically raise questions such as: Is it possible to market new inventions immediately at costs as low or lower than those of conventional equipment? Is it not generally true that corporations marketing a new product set up "promotional costs" as a legitimate expense of promoting a new product during its first few months? Also, that frequently such costs are continued during the first 2 or 3 years after the equipment appears on the market, during which time the product is marketed below cost? If the cost-plus basis of cost accounting of all companies had been used as the basis for establishing minimum prices during the past 50 years, would it have been possible to introduce new inventions such as the electric light (competing with gas lights) and the electric refrigerator (competing with the ice refrigerator)?

Fortunately from the public viewpoint, monopolistic exactions which curb invention and

discovery eventually are caught up with. A case in point is to be found in a decision of the Virginia Supreme Court. The case arose out of the fact that the Richmond Milk Commission established the price for milk sold in paper containers 1 cent a quart higher in the Arlington-Alexandria milk marketing area than the price when sold in glass bottles. A dealer in that area who wanted to sell milk packaged in paper petitioned the Milk Commission to amend or set aside the requirement.

The Virginia Supreme Court in a decision handed down March 13, 1944, stated:[3] "The Commission has the power to fix different prices for different grades of milk. It has not been given the power to fix a different price for the same grade of milk. . . . For the foregoing reasons we are of the opinion that the regulation under review is invalid insofar as it undertakes to establish a difference in price for the same grade of milk based upon the type of container in which it is sold." Since this decision was made, the Massachusetts Supreme Court has handed down a similar decision. Presumably, the long-time effect of the decision of these supreme courts will be to remove the exaction of higher price for milk sold in paper containers in the other eastern states where this ruling exists. In March, 1955, however, five states with state-regulated consumer prices —Georgia, Maine, Montana, New Hampshire, and Rhode Island—still exacted a higher price for milk in paper containers than for milk in glass bottles.

Other requirements, equally questionable, are still found under state-controlled legislation. For example, California prohibits the use of the gallon jug on the ground that it is unsanitary. One may wonder if the 1,600,000 consumers buying Grade A milk in gallon jugs in Chicago are buying a product packaged in unsanitary containers. Or does it mean that the California restriction against the gallon jug is an artificial regulation put in by vested interests to protect themselves against competition? Many states, including New York,[4] have similar regulations against the use of the gallon jug.

[3] *The Milk Industry* (Bartlett), Ronald Press, 1945, pp. 81 to 87.

[4] A bill is now (March, 1955) before the New York legislature to permit the use of gallon jugs.

Should Milk Control Boards Legalize Inefficiency?

The decision reached by the Virginia Supreme Court was a move in the right direction in letting up on exactions which curb the flow of milk to the consumer. But as part of this decision, the Supreme Court made another statement, which should be considered carefully in view of its general acceptance in the realm of fixing of consumer prices. The Court went on to state: "The Act was not intended to discourage the installation of new machinery, the employment of new processes, or the practice of economical operation. Rather it was intended to encourage them, both as a benefit to the producer and the consumer, thereby favoring the public weal. If a producer and distributor using fiber or paper container is satisfied to sell his milk at the same price as a competitor who uses glass containers, the consumer who prefers the fiber or paper container has no complaint. So long as the producer and distributor who uses the bottles as containers makes a fair profit, they have no just grievances. There is nothing unfair, unjust, or demoralizing in either trade practice."

The regulation of public utilities by law is based on the protection of the consumer from unjust or exorbitant rates but is also predicated upon allowing the utility a fair profit on its investment and operation of its plant. A milk dealer falls into the same category when the state controls consumer prices. If margins are lowered in line with the most efficient group of distributors, a dealer can allege "confiscation" and appeal to the "due process" provision of the Constitution. This tends to limit the usefulness of governmental price control in obtaining increased efficiency.

Should we accept the theory that every dealer, efficient or inefficient, be permitted to make a fair profit? If so, where would this theory lead us over a period of years? If the automobile industry had adopted that theory back in 1900, would automobiles have come into common use, with one car for every four people or would they still be a luxury with only one car per 1,000 or more people, as exists in most of Europe?

According to R. C. Epstein, in his book *Automobile Industry,* there were 181 companies manufacturing automobiles between 1895 and 1927. In 1927 only 44 of the companies were still in business. Thus, 137 companies during this period, for some reason or other, went out of business. And since 1927 there have been many other mergers. The motor vehicle industry constitutes one of the best examples of the development of our system of free enterprise. The government did not and could not decide which of the companies would stay in business and which fail. This decision was made by competition, and as consumers we are reaping the benefits of risk-taking by the companies who survived.

Governmental fixing of consumer prices has tended to curb the use of inventions and to legalize inefficiency in the distribution of milk. Fourteen states, as well as the federal government, have already discontinued this program. From the viewpoint of the best public interest, it is to be hoped that legislators in the states still having this type of regulation will take steps to restore competitive pricing at the consumer level.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

334. The histopathology of bovine mastitis. C. F. HELMBOLDT, E. L. JUNGHERR, and W. N. PLASTRIDGE, Storrs Agr. Expt. Sta., Storrs, Conn. Bull. 305. 1953.

This study is based upon bacteriologic and pathologic data on bovine udders collected during the period from Nov. 1930 to Nov. 1950. A total of 138 udders were available for histologic study. The bulletin is a technical presentation of the findings, including several pictures of udders examined. R. W. Hunt

335. Bovine mastitis II. A study of the underlying causes of mastitis and evaluation of various measures that may be taken to effect control. J. J. REID, Penn. Agr. Expt. Sta., State College. Bull. 581. 1954.

Any measures which are undertaken to destroy microorganisms associated with the disease are ineffectual. Data show that there is no inherent danger in placing healthy animals next to animals infected with *Streptococcus agalactiae*. The bacteria associated with mastitis take advantage of existing pathological conditions rather than creating such a condition themselves. Investigations of the fundamental causes of this disease showed that both heredity and environment are involved. Animals of different heredity may vary greatly in relative susceptibility to udder trouble. Environmental factors involved in the development of mastitis include poor milking practices, improperly operated milking machines, poor housing, lack of bedding, exposure to inclement weather, mechanical injuries, improper feeding practices, improper drying technique, and lack of adequate care of the dry and freshened animal.

R. W. Hunt

336. Bovine mastitis II. A study of the underlying causes of mastitis and evaluation of various measures that may be taken to effect control. J. J. REID, Penn. Agr. Expt. Sta., State College. Bull. 581-P. 1954.

This bulletin presents the practical conclusions contained in Penn. Agr. Expt. Sta. Bull. 581. R. W. Hunt

BOOK REVIEWS

337. Experimental Cookery—From the Chemical and Physical Standpoint. By Belle Lowe. 4th ed. John Wiley & Sons, New York. 1955. 573 pp. $7.50.

The author presents the chemical and physical basis of food preparation in a form suitable for use as a reference book on cookery.

The fundamental principles of colloid chemistry involved in cookery are discussed and the second chapter is entirely new and deals with food acceptability. The section entitled "Methods of Food Evaluation" should prove helpful in any serious study of food quality.

The book abounds in suggested problems and experiments covering a wide range of food preparation, freezing as well as cooking. The extensive bibliography is well chosen and made more usable by classifying many of the references under such headings as ice cream, frozen fruit and vegetables, frozen meat and poultry, and frozen bread, batter products, and cake. The many photographs and charts are helpful in understanding the chemical and physical principles involved in food preparation. This revised edition, as were the previous editions, will be useful to those interested in the scientific aspects of food preparation. Lita Bane

338. This Is Liquid Sugar. Refined Syrups & Sugars, Inc. Yonkers, N. Y. 1955.

The nine chapters of the manual are as follows: I. What Liquid Sugar Is; II. History of Liquid Sugar; III. Production of Liquid Sugar; IV. Distribution of Liquid Sugar; V. Equipment Used in Liquid Sugar Systems; VI. Liquid Sugar in Major Industries; VII. Engineering Services; VIII. Laboratory Procedures; IX. Technical Data.

A five-part appendix consists of A—Types of "Flo-Sweet" Liquid Sugars, B—Liquid Sugar Pricing, C—Cane Refiners' Spreads, D—The United States Sugar Act, E—Glossary.

A number of illustrations, charts, graphs, and diagrams supplement the text throughout the manual. L. M. Dorsey

BUTTER

339. Butter handling apparatus. W. Massmann (assignor to Benz & Hilgers Maschinenfabrik). U. S. Patent 2,705,835. 3 claims. Apr. 12, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 2: 149. 1955.

A combination worm and piston extruding device for packaging butter. R. Whitaker

340. Packaging of fats. J. K. Heidrich and C. D. Mullinix (assignor to C. D. Mullinix). U. S. Patent 2,705,203. 7 claims. Mar. 29, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 5: 625. 1955.

A carton so designed that chips or individual pats of butter may be removed easily through a slot without unwrapping the product.

R. Whitaker

CHEESE

341. How sanitary is your cottage cheese? P. R. Lyons, Lansing-Ingham County Health Dept. and W. L. Mallmann, Mich. State Coll., East Lansing. Milk Plant Monthly, **44**, 3: 17. 1955.

Data are presented showing: (1) The coliform incidence of cottage cheese packaged at the plant; (2) The coliform incidence in cottage cheese in bulk at retail stores; (3) Comparative coliform incidence in cottage cheese processed and packed by hand and mechanically, and (4) The coliform incidence in the various stages of processing and packaging cottage cheese. The data show that about 67% of the samples collected contained coliforms in the amout of 220 or more per 100 g. cheese. The percentage of positive samples collected from each plant varied from 33 to 100%. There seemed to be no correlation between the individual plant's ability to produce coliform-free bottled products and its ability to produce coliform-free cottage cheese. Mechanization of all steps in the processing and packaging of cottage cheese can do much toward eliminating this contamination. C. J. Babcock

CONDENSED AND DRIED MILKS; BY-PRODUCTS

342. Army approves concentrated milk. R. G. McCall, Lt. Col., MSC, Army Environmental Health Lab., AMS. Milk Plant Monthly, **44**, 4: 20. 1955.

Soldiers reported no preference for whole pasteurized milk over a new reconstituted milk concentrate after a recent three-months' study at Ft. Bliss, Texas. The study showed that "three-to-one" concentrated milk can be satisfactorily shipped from surplus areas to a military camp several thousand miles away for reconstitution, pasteurization, and packaging at a fluid milk plant. Some conclusions to the study are: (1) The concentrate had an excellent keeping quality for 9-10 days and an acceptable keeping quality through the holding period of 15 days, (2) The dairy reconstituted milk had a bacterial and chemical quality equal to whole pasteurized milk, (3) The dairy-reconstituted milk had a three-day keeping quality equivalent to that of whole pasteurized milk, and (4) Reconstitution by the "pitcher" method is unsatisfactory due to an increase in bacteria count, the possibility of chance contamination and loss of six % volume of the finished product. Conditions under which the National Research Council concurred in the use of 3-1 concentrated milk are set forth. C. J. Babcock

343. Foremost introduces "Fresh Process" evaporated milk. Anon. The Milk Prod. J., **46**, 4: 19. 1955.

Foremost Dairies, Inc. has introduced a new "Fresh Process" evaporated milk on the West Coast market. Basically, the processing function is filling and sealing a sterile product in a sterile container in a sterile atmosphere. The Dole aseptic canning system is part of the process.

The aseptic canning unit sterilizes the cans with superheated steam as they are conveyed continuously to the filler. The can covers also are sterilized by superheated steam by a sterilizer attached to and operated as an accessory portion of the closing machine.

An atmosphere of superheated steam (or hot inert gas) is maintained in the filler, closing machine, and the interconnecting conveyor system to maintain sterility and prevent the entry of air-borne bacteria into the system.

The product has been distributed for several months throughout the San Francisco Bay region. J. J. Janzen

DAIRY BACTERIOLOGY

344. Respiration rate of bacteria as a function of oxygen concentration. I. S. Longmuir, Microbiol. Research Dept., Porton, Wilshire. Biochem. J., **57**, 1: 81. 1954.

A polarographic method is described for measuring and continuously recording the concentration of oxygen in solution down to concentrations of the order of 10^{-9} M. This method was used to determine the respiration rate of a number of species of bacteria as a function of oxygen concentration. Results obtained indicated that the respiration rate of these bacteria was related to oxygen concentration by the Michaelis-Menten equation. The characteristic K_m (concentration of oxygen at which bacteria respire at half their maximum rate) was determined. The K_m was found to increase markedly with increase in the temperature from the neighborhood of 20° C. to 35° C. With one organism at least *(Aerobacter aerogenes)*, K_m did not differ appreciably when the pH was shifted from 7.3 to 9.0. It is suggested that K_m is related to the size of the organism.

R. Jenness

345. The utilization of non-protein nitrogen in the bovine rumen. 8. The nutritive value of the proteins of preparations of dried rumen bacteria, rumen protozoa and brewers' yeast for rats. M. L. McNaught and E. C. Owen, Hannah Dairy Research Inst., Kirkhill, Ayr, and K. M. Henry and S. K. Kon, Natl. Inst. for Research in Dairying, Univ. of Reading. Biochem. J., **56**, 1: 151. 1954.

This paper reports a determination of biological value and true digestibility of the bacterial and protozoal proteins prepared from bovine rumen contents. A sample of dried bacteria was prepared from bovine rumen liquid and a sample of dried protozoa from rumen contents by straining them through muslin, diluting the strained liquid with glucose solution and allowing this liquid to settle at 39° C. The protozoal layer was separated by decantation. The dried bacteria and protozoal materials contained respectively 41.8% and 26.5% of crude protein. The biological value and true digestibility of these samples and of dried Brewer's Yeast were determined at an 8% level of protein intake on groups of 12 young female rats by the balance sheet method. Biological values of 81, 80, and 72% were obtained respectively for the proteins of bacteria, protozoa, and yeast. The corresponding values for true digestibility were 74, 91, and 84%. These results suggest that the conversion of bacterial or dietary protein into protozoal protein in the rumen is advantageous to the host animal.

R. Jenness

346. The extent of conversion of food protein to microbial protein in the rumen of sheep. I. W. McDonald, Agr. Research Council, Inst. of An. Physiol., Babraham Hall, Cambridge. Biochem. J., **56**, 1: 120. 1954.

When sheep were fed a partially-purified diet to which zein contributed 94% of the total nitrogen, it was found that approximately 40% of the zein was utilized by ruminal microorganisms for the synthesis of their own proteins. This experiment involved the collection of the digesta received by the duodenum and separation of the ethanol-soluble protein zein from microbial protein therein. The conclusions reached with this experiment were supported by another based upon analyses of lysine in the foodstuffs, ruminal microorganisms and abomasal contents. The assumptions involved in this work are discussed and reasons are suggested for the belief that in normal dietary regimes a higher proportion of the food protein might be converted in the rumen into microbial protein than in the case of this experiment.

R. Jenness

347. Amino acid utilization in bacterial growth. 2. A study of threonine-isoleucine relationships in mutants of Escherichia coli. H. Amos and G. N. Cohen, Institut Pasteur, Garches, France. Biochem. J., **57**, 2: 338. 1954.

The growth of four mutants of *Escherichia coli* that require L-threonine was found to be inhibited by the presence of L-serine in the culture medium. The inhibition by DL-serine was competitive and was suppressed by D-threonine, α-aminobutyric acid, α-oxobutyric acid, α-oxo-β-hydroxybutyric acid, and L-isoleucine. All of these compounds except L-isoleucine exerted a sparing effect in the utilization of L-threonine. A mutant requiring either D-threonine, α-aminobutyric acid or L-isoleucine is also inhibited by DL-serine when grown on D-threonine or α-aminobutyric acid. From these results it is suggested that L-isoleucine synthesis depends on L-threonine utilization in these mutants.

R. Jenness

348. Stoicheiometry of the citrulline phosphorylase reaction of Streptococcus lactis. M. Korzenovsky and C. H. Werkman, Dept. of Bacteriol., Iowa State Coll., Ames. Biochem. J., **57**, 2: 343. 1954.

A complete chemical balance of the products of the citrulline phosphorylase reaction of *Streptococcus lactis* is presented. The data show that 1 mole of orthophosphate was required to degrade one equivalent of citrulline to ornithine, carbon dioxide, and ammonia. One equivalent of ATP was synthesized. Extracts of *S. lactis* release orthophosphate from ATP but not from ADP. The presence of an adenylate kinase system is indicated. R. Jenness

349. Enzymatic synthesis of a growth factor for Lactobacillus bifidus var. Penn. F. Zilliken, P. N. Smith, C. S. Rose, and P. György, Univ. of Penn., Philadelphia. J. Biol. Chem., **208**: 299. 1954.

Two isomeric disaccharides of galactose and N-acetyl-D-glucosamine were synthesized from lactose and N-acetyl-D-glucosamine by means of an enzyme preparation from *L. bifidus* var. *Penn.* These studies were prompted by the demonstration in human milk previously of N-acetyl-D-glucosamine containing oligosaccharides which promote the growth of *L. bifidus* var. *Penn.* Only one of the isomers isolated was quite active in the microbiological test.

B. L. Larson

350. Studies on canned cheese spread experimentally inoculated with spores of Clostridium Botulinum. R. O. Wagenaar and G. M. Dack, Food Research Inst., Univ. of Chicago, Ill. Food Research, **20**, 2: 144. 1955.

A cheese spread of the type used in military field rations containing 35 to 37.5% moisture, 1.25 to 2.50% salt, a high fat level, at pH 5.2 to 6.2, did not support the growth or toxin production when inoculated (10,000/g.) with *C. botulium* spores and held in cans at 90° F. for periods up to six months. All samples, however, contained viable spores, but in smaller numbers, at the end of the period.

F. J. Doan

351. The probable extent of starter failure due to residual antibiotics. R. W. Hennings-son, Dept. of Dairy Ind., Cornell Univ. Ithaca, N. Y. The Milk Prod. J., **46**, 3: 64. 1955.

In the last four years separate surveys have been conducted in New York State, Canada, and England. The New York state samples were pooled milk from several herds while the Canadian samples were individual herd milks. The English samples were pooled herd samples and individual can samples representing a part of a herd. Both the Canadian and New York state surveys measured the total inhibition by means of a modified starter activity test and the antibiotic inhibition by assay in terms of penicillin. The English survey measured only antibiotic inhibition by assay in terms of penicillin.

The authors of the three surveys independently arrived at the general conclusion that at the time of the surveys there existed no grave problem in the areas examined as far as the manufacturer of any fermented milk products was concerned. They agree that in isolated cases an individual manufacturer may encounter serious trouble. J. J. Janzen

352. The problem of false coliform counts on fruit ice cream. F. W. Barber and H. Fram, Natl. Dairy Research Lab., Inc., Oakdale, Long Island, N. Y. J. Milk and Food Technol., **18**, 4: 88. 1955.

Studies indicate that there are other microorganisms in fruit ice cream besides coliform bacteria that may give a positive test. Obviously some reservation in the interpretation of the coliform test in fruit ice cream should be observed. H. H. Weiser

353. Mold control in bakeries. W. Reeder, Campbell Taggart Research Corp., Dallas, Texas. J. Milk and Food Technol., **18**, 4: 90. 1955.

The spoilage of bread by molds is a significant problem in homes and in industry. Since the spores are widely distributed mold control requires careful engineering and good sanitary practices. The emphasis should be on controlling moisture, temperature, pH, good sanitation and the use of inhibitors of mold growth. H. H. Weiser

DAIRY CHEMISTRY

354. The biosynthesis of proteins. 2. Synthesis of milk proteins by the goat. B. A. Askonas, P. N. Campbell, and T. S. Work, Natl. Inst. for Med. Research, Mill Hill, London. Biochem. J., **58**, 2: 326. 1954.

A lactating goat was injected with radioactive glycine, valine, lysine, and methionine and the appearance of radioactivity in the milk proteins was followed. After injection of methionine-S^{35} both casein and the whey proteins reached maximum activity in about 1.5 hours. The whey activity curve followed closely the casein curve but was always slightly below it. Casein and crystalline β-lactoglobulin were isolated from the milk obtained after simultaneous injection of glycine-2-C^{14}, DL valine-4-C^{14}, and DL lysine-1-C^{14}. These proteins were hydrolyzed and the radioactive amino acids isolated as the dinitrophenol derivatives. The radioactivity of the glycine, valine, and lysine from casein did not differ significantly from that of the corresponding amino acids from β-lactoglobulin. The casein serine, however, was more active than the β-lactoglobulin serine. These results suggest that the nitrogen pool for the synthesis of casein and β-lactoglobulin is identical and that this pool is in equilibrium with free circulating amino acids. R. Jenness

355. Phospholipids. 2. Estimation of amino nitrogen in intact phospholipids. C. H. Lea and D. N. Rhodes, Univ. of Cambridge. Biochem. J., **56**, 4: 613. 1954.

The colorimetric ninhydrin procedure of Moore and Stein was modified to maintain lipids in solution and was applied to the estimation of phosphatidylethanolamine (1 to 5 micrograms nitrogen) in the presence of phosphatidylcholine (up to 1,000 micrograms N) without preliminary hydrolysis of the protein. Strict conformity with Beer's Law and satisfactory reproducibility were easily obtained within any one run but considerably different color yields could result in different runs with the same substance unless care was taken with the purification and storage of the reagent and particularly with the elimination of peroxide from the cellosolve that was used as the solvent for the phospholipids. Phosphatidylcholine as well as phosphatidylethanolamine was found to liberate gas in the Van Slyke manometric procedure for the determination of amino nitrogen owing to a reaction of its component unsaturated fatty acids with nitrous acid. Thus, the Van Slyke method is only suitable for use in an empirical manner using a reaction time chosen for the particular material to agree with a determination on the fatty acid-free hydrolysate. The Van Slyke method is much less sensitive than the ninhydrin method for determining amino nitrogen in phospholipids. R. Jenness

356. The branched-chain fatty acids of butterfat. 4. The isolation of (+)-12-methyltetradecanoic acid and of 13-methyltetradecanoic acid. R. P. Hansen, F. B. Shorland, and N. J. Cooke, Fats Research Lab., Dept. of Sci. and Ind. Research, Wellington. N. Z. Biochem. J., **57**, 2: 297. 1954.

Two branched-chain fatty acids (+)-12-methyltetradecanoic acid and 13-methyltetradecanoic acid formerly isolated from mutton fat have been shown to be present in butterfat. The amounts of these two C_{15} acids together constitute about 0.1% of the total fatty acids present in the butterfat. R. Jenness

357. The branched-chain fatty acids of butterfat. 5. The isolation of 12-methyl tridecanoic acid. R. P. HANSEN, F. B. SHORLAND, and N. J. COOKE, Fats Research Lab., Dept. of Sci. and Ind. Research, Wellington, N. Z. Biochem. J., **58**, 3: 358. 1954.

12-Methyl tridecanoic acid was isolated in trace amounts (0.05% of the total fatty acids) from butterfat by methods which included hydrogenation, fractional distillation and low temperature crystallization. R. Jenness

358. Electrophoretic studies of ox serum. 1. The sera of normal cattle. C. J. BRADISH, W. M. HENDERSON, and J. B. BROOKSBY, Research Inst., Pirbright, Surrey. Biochem. J., **56**, 2: 329. 1954.

The blood sera of 51 normal Devon steers from 18 to 30 months old were analyzed electrophoretically in a phosphate buffer of pH 7.6 and ionic strength 0.18. Several other buffers were tried but the one indicated gave the best resolution in this study. The mean electrophoretic distribution was found to be: albumin—46.6%, α-globulin—14.0%, β-globulin—8.9%, and γ-globulin—30.5%. The variability among animals is presented in the form of frequency distributions. The γ-globulin was found to consist of at least three components. The data for 30 of these steers indicated an absolute concentration of protein in the serum of 7 ± 0.5 g. per 100 ml. A significant correlation was found to exist between individual values for the serum protein concentration and the electrophoretic distribution. The absolute concentration of serum albumin was relatively constant and close to 3.2 g. per 100 ml. despite much greater changes in the absolute concentration of the serum globulins. R. Jenness

359. Electrophoretic studies of ox serum. 2. The sera of cattle infected with the virus of foot-and-mouth disease. C. J. BRADISH, W. M. HENDERSON, and J. B. BROOKSBY, Research Inst., Pirbright, Surrey. Biochem. J., **56**, 2: 335. 1954.

The blood sera of 21 Devon steers were analyzed electrophoretically during the course of infection with the virus of foot-and-mouth disease. Six strains of virus were employed and a regular development of the electrophoretic distribution was observed in all but one of the 21 series of sera. The same electrophoretic techniques were used as in the previous paper but a closed electrode vessel is described for the manipulation of ineffective solutions. In the serum of the "average, typical animal," the β-globulin rose from the normal level of 10% to about 14% on the seventh day following inoculation. The normal level of β-globulin was regained during the following week. The γ-globulin component of the serum increased from the normal level of 30% to about 40% during the third or fourth week and maintained this value with little change for several weeks. The γ-globulin component in bovine serum comprises at least three components which have been characterized by their mobility ranges. In infection the observed increase in γ-globulin occurred in the components of lowest mobility. R. Jenness

360. Electrophoretic studies of ox serum. 3. The sera of cattle infected with the virus of vesicular stomatitis. C. J. BRADISH and J. B. BROOKSBY, Research Inst., Pirbright, Surrey. Biochem. J., **56**, 2: 342. 1954.

The blood sera of four cattle were analyzed electrophoretically during the course of infection with two strains of the virus of vesicular stomatitis. An increase in the γ-globulin level of from 3 to 10% above normal was observed between the third and sixth week following inoculation. In a second experiment, inoculation of two cattle with the same strain of virus given 31 days after the first inoculation failed to induce any significant change in the electrophoretic distributions of the serum proteins collected within the following 22 days. Of two animals which received a second inoculation of a different type of virus 53 days after the first, the serum of only one showed a significant rise in the proportion of γ-globulin during the following 22 days. These data suggest that the electrophoretic changes in bovine serum associated with the development of vesicular stomatitis are less pronounced and less regular than those associated with foot-and-mouth disease. R. Jenness

361. The interaction of dyes with proteins on paper with special reference to paper electrophoresis. G. T. FRANGLEN and N. H. MARTIN, Dept. of Chem. Path., St. George's Hospital Med. School, London. Biochem. J., **57**, 4: 626. 1954.

The spectral absorption characteristics of the dyes bromophenol blue, bromocresol green, azocarmine B, and naphthalein black 12B were examined. Azocarmine B was unsatisfactory for chromatography work as it is heterogeneous upon chromatography on filter paper. Bromocresol green was superior for dye for quantitative work since it did not fade like bromophenol blue and naphthalein black 12B. The dye-binding characteristics of human albumin and human γ-globulin with bromocresol green were studied individually and in mixtures of known composition. The plot of the dye bound to protein against the concentration of protein was not linear. Moreover, the characteristics of the relation of dye binding to protein concentration differed from one protein to another. Finally when the two proteins were mixed in predetermined proportions and then separated by paper electrophoresis the proportions calculated by dye-binding techniques failed to tally with the pre-determined ratios. These results suggest that existing quantitative methods of analysis by paper strip electrophoresis need reassessment. R. Jenness

362. The estimation of lactic acid using ceric sulphate. S. R. Elsden and Q. H. Gibson, Dept. of Microbiol. and Dept. of Physiol., Univ. of Sheffield. Biochem. J., **58**, 1: 154. 1954.

The effects of ceric sulphate concentration and temperature on the oxidation of lactic acid by ceric sulphate were investigated. Significant amounts of the acetaldehyde produced are oxidized to lactic acid if the acetaldehyde is not removed rapidly from the reaction mixture. The rate of destruction of acetaldehyde is related to the concentration of ceric sulphate in the reaction mixture and of the temperature. Consequently, to obtain maximum recovery of acetaldehyde, the concentration of ceric sulphate should be such as to insure only a slight excess at the end of the reaction. The rate of aeration should be the maximum possible. A new method for the estimation of lactic acid using ceric sulphate and removal of the acetaldehyde by steam distillation is described.

R. Jenness

363. The application of four methods for assessing protein homogeneity to crystalline β-lactoglobulin: An anomaly in phase rule solubility tests. O. Smithies, Dept. of Chem., Univ. of Wis., Madison. Biochem. J., **58**, 1: 31. 1954.

The homogeneity of two preparations of β-lactoglobulin was assessed by the following four methods: ultracentrifugation, immunological test (precipitation curve determination), electrophoresis, and a variable solvent solubility test. The ultracentrifugal and immunological methods employed were unsatisfactory as they showed no evidence for inhomogeneity in the sample tested. The electrophoretic studies indicated the presence of at least two components in a lactoglobulin sample but marked anomalies occurred which rendered the method unsatisfactory as a means for quantitatively determining the proportion of components. The variable solvent solubility test employing varying concentrations of ammonium sulfate was the most satisfactory of the methods evaluated. It indicated the presence of two main components in a ratio of approximately 1:4 in lactoglobulin at pH's of 4.5, 4.8, and 5.1. Repeated recrystallizations did not affect this ratio. At pH 5.4 an additional discontinuity occurred in the solubility curve for lactoglobulin associated with a different form of the solid phase. It was concluded that this does not represent a true additional component in the protein. At pH 5.7 the solubility behavior of β-lactoglobulin depended markedly upon time. These results were shown to be due to progressive crystallization. This appears to be the first time that such anomalies in phase rule solubility tests have been demonstrated experimentally. The ways of detecting anomalous behavior of this type are discussed.

R. Jenness

364. Crystallization of goat lactoglobulin. B. A. Askonas, Natl. Inst. for Med. Research, Mill Hill, London. Biochem. J., **58**, 2: 332. 1954. **Addendum: Diffusion coefficient of crystalline goat β-lactoglobulin.** E. A. Caspary, Lister Inst. for Preventive Med., London. Ibid. pg. 336.

β-Lactoglobulin from goat milk was crystallized from ammonium sulphate solution at approximately 0.6 to 0.65 saturation. The crystals were well-formed hexagonal bipyramids. Attempts at crystallization of the goat β-lactoglobulin by dialyzing the preparation free from salt at its isoelectric point (the isoelectric point of this protein was pH 5.9) at 0° for one to two weeks was unsuccessful although an oily layer separated. The electrophoretic behavior of the goat β-lactoglobulin was studied at pH values ranging from 4.1 to 8.1. The protein appeared homogeneous forming a symmetrical boundary at pH 4.15 and at 5.6 to 8.1. An asymmetric boundary was noted in the descending limb at pH 4.6 and 5.1 in sodium acetate buffer, ionic strength 0.1. The sedimentation constant S_{20} for this protein at pH 6.9, ionic strength 0.2 was found to be 2.85 S and was independent of concentration from 0.2 grams per 100 ml. to 1 gram per 100 ml. The diffusion constant D_{20} was 7.48×10^{-7}. The molecular weight was calculated to be 37,100.

R. Jenness

365. Derivatives and kinetics of lactoperoxidase. D. B. Morell, Molteno Inst., Univ. of Cambridge. Biochem. J., **56**, 4: 683. 1954.

A method of measuring the activity of lactoperoxidase, depending upon the increase in optical density at 300 mμ using pyrogallol as a hydrogen donor is described. Conditions of measurement were chosen such that with high concentration of the donor and low concentration of peroxide reaction of the enzyme with peroxide was measured. A method is described for purifying the lactoperoxidase to an extent of 40-50% absolute purity. Absorption curves were determined for lactoperoxidase and its carbon monoxide, cyanide, fluoride, and pyridine derivatives. The absorption spectra are very similar in pattern to those for the protohematin compounds horseradish peroxidase, catalase, and methemoglobin. This suggests that the prothetic group of lactoperoxidase is probably hematin closely related to protohematin but it probably is not protohematin itself since the absorption bands lie further toward the longer wavelengths. Data are presented on the kinetics of the action of lactoperoxidase and the Michaelis constant was determined experimentally to be 1.08×10^{-3} M.

R. Jenness

366. Chromatographic determination of cystine as cysteic acid. E. Schram, S. Moore, and E. J. Bigwood, Lab. of Biochem., Fac. of Med., Univ. of Brussels. Biochem. J., **57**, 1: 33. 1954.

A method is presented for the determination of cystine plus cysteine in proteins. This method is based upon oxidation of the protein by performic acid, hydrolysis by hydrochloric acid, and chromatographic determination of the resulting cysteic acid on columns of a basic ion-exchange resin (Dowex-2). This method avoids complications due to instability of cystine and cysteine during protein hydrolysis, particularly in the presence of carbohydrate, since both cystine and cysteine residues in the intact protein are converted into cysteic acid which is stable under conditions of acid hydrolysis. Data secured with the method are presented for a number of proteins, including casein, and the method was also applied to a number of food products, including milk.

R. Jenness

367. The determination of the optical configuration of naturally occurring amino acids using specific enzymes and paper chromatography. E. Bonetti and C. E. Dent, Univ. Coll. Hospital, London, W.C. 1. Biochem. J., **57**, 1: 77. 1954.

A micromethod is described for determining the optical configuration of amino acids in biological fluids. It involves incubation of the fluid with D-amino acid oxidase of sheep's kidney and L-amino acid oxidase of snake venom. The extent of the reactions produced by these enzymes is followed by paper chromatographic analysis. The method was applied to acid hydrolysate of casein, to human plasma ultrafiltrate, to cerebrospinal fluid, and to urine. No evidence was obtained of the presence in these fluids of the D forms of any of the ten amino acids for which the method is applicable at present (these ten amino acids are alanine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophane, tyrosine, and valine).

R. Jenness

368. The purification of alkaline phosphatases of animal tissues. R. K. Morton, Dept. of Biochem., Univ. of Cambridge. Biochem. J., **57**, 4: 595. 1954.

The alkaline phosphatases of calf intestinal mucosa and of cow mammary gland were shown to be associated with microsomal particles (particles of 200-20 mμ prepared by differential centrifugation). Treatment with n-butanol at 35° C. releases the enzyme into true solution. A method for assaying of alkaline phosphatase in animal tissues is suggested based upon this effect. The purification of alkaline phosphatase from calf intestinal mucosa is described. The procedure involves butanol treatment of washed microsomes, acetone precipitation, heating, and charcoal adsorption. The most active preparation obtained had an activity (with β-glycerophosphate) of 83,500 units per mg. N. The alkaline phosphatases of kidney, liver, and mammary gland were partially purified by similar methods.

R. Jenness

369. The estimation of serum proteins by electrophoresis. J. Hardwicke, Dept. of Exptl. Path., Univ. of Birmingham. Biochem. J., **57**, 1: 166. 1954.

A method is described for quantitative electrophoresis of proteins on filter paper. A number of variables inherent in the method were examined critically. These include staining and washing of the papers, reproducibility of the staining, optimum concentration of protein solutions, type of paper, tailing of proteins on the paper during migration and dye-binding capacity of different protein fractions. Recovery experiments and duplicate determinations indicated the quantitative accuracy of the method adopted to be ±6% of the total protein present.

R. Jenness

370. A micromethod based on zone electrophoresis and cholesterol estimations. G. S. Boyd, Dept. of Biochem., Univ. of Edinburgh, Scotland. Biochem. J., **58**, 4: 680. 1954.

A micromethod is described for the separation and estimation of the serum lipoproteins. This procedure uses only .1 to .2 ml. of serum and it involves filter paper zone electrophoresis followed by segmentation of the supporting paper, elution of cholesterol and estimation of the latter by a colorimetric micromethod. Two lipoproteins, α and β, are observed and summation of the cholesterol data permits calculation of the ratio of these two components.

R. Jenness

371. The source of antibody globulin in rabbit milk and goat colostrum. B. A. Askonas, P. N. Campbell, J. H. Humphrey, and T. S. Work, Natl. Inst. for Med. Research, Mill Hill, London, N.W. 7. Biochem. J., **56**, 4: 597. 1954.

In the first experiment a lactating rabbit was immunized with pneumococcus (type III) and was then given an injection of methionine-S^{35}. Blood and milk samples were withdrawn at intervals and a specific polysaccharide was used to precipitate the specific antibody from blood and milk. The antibody concentration in the milk was about 1/20th of that in the blood. The serum antibody and the milk antibody acquired a similar degree of radioactivity. Casein and whey protein (after removal of antibody) collected six hours after injection of methionine had six to ten times the radioactivity of milk or serum antibody collected at the same time. In a second experiment a goat was injected with methionine-S^{35} shortly after parturition and the colostrum samples taken at 10 and 13 hours after the injection were separated into casein, an immune globulin fraction and "lactoglobulin" (actually the "immune globulin" fraction represented that protein precipitated at 0.49 saturation at pH 4.6 and the "lactoglobulin" fraction that precipitated between 0.49 saturation and 1.00 saturation at the same pH). The casein and "lactoglobulin" fractions ac-

quired much more radioactivity than the immune globulin. These results indicated that in both the rabbit and the goat antibody protein passes from the blood into the milk or colostrum without degradation and resynthesis.

R. Jenness

372. Purification and properties of phosphoprotein phosphatase from ox spleen. T. A. SUNDARARAJAN and P. S. SARMA, Univ. Biochem. Lab., Madras 25, India. Biochem. J., **56**, 1: 125. 1954.

A phosphatase capable of liberating inorganic phosphate from phosphoprotein was shown for the first time to be present in cattle tissues. A procedure was developed for partial purification of this enzyme from ox spleen. Using a combination of ammonium sulfate and acetone fraction procedures, the enzyme was concentrated 200-fold with an over-all yield of about 20%. A purified preparation of the enzyme attacked three phosphoproteins that were studied: casein, phosphovitin, and vitellin. However, these preparations had no appreciable action on glycerolphosphate or on a phosphopeptone prepared from casein. Thus, the enzyme is a true phosphoprotein phosphatase distinct from phosphomonoesterase. This enzyme exhibited optimum activity at pH 6 and at a substrate concentration corresponding to about 10 micromoles of casein phosphorus per ml. Activation and inhibition studies indicated that sulfhydryl and amino groups are essential for the activity of this enzyme but it requires no dialyzable co-enzyme for activity.

R. Jenness

373. Paper-chromatographic analysis of Dakin's samples of "8-hydroxyglutamic acid." C. E. DENT and D. I. FOWLER, Med. Unit, Univ. Coll. Hospital, London, W.C. 1. Biochem. J., **56**, 1: 54. 1954.

It was claimed by Dakin (Biochem. J. **12**: 290. 1918, and **13**: 398. 1919) that proteins contained β-hydroxyglutamic acid. Later work has thrown doubt on this claim. This paper reports an investigation by both one-way and two-way chromatography of two samples of material isolated from protein hydrolysates (one of them from casein) by Dr. Dakin himself and alleged by him to be β-hydroxyglutamic acid. No hydroxyglutamic acid was found in either sample. Both samples were found to consist of mixture of commonly occurring amino acids, mostly glutamic and aspartic acids.

R. Jenness

374. The effect of p-nitrophenol on the Pasteur reaction and on aerobic phosphorylation in suspensions of the mammary gland. C. TERNER, Natl. Inst. for Research in Dairying, Univ. of Reading. Biochem. J., **56**, 3: 471. 1954.

A Pasteur effect coupled with aerobic phosphorylation was demonstrated in cell-free suspensions of mammary gland incubated with fructose 1,6-diphosphate in the presence of glucose and yeast hexokinase as the phosphate acceptor system. These phenomena were inhibited by *p*-nitrophenol at a concentration of $2\text{-}4 \times 10^{-4}$ M which at the same time could cause up to 50% stimulation of respiration. *p*-Nitrophenol had no effect on respiration of the suspensions in the absence of added substrates. The Pasteur effect also was shown to occur in mammary suspensions incubated with fructose 1,6-diphosphate in the absence of added phosphate acceptors. In these experiments the initial level of inorganic phosphate was approximately maintained under aerobic conditions. The addition of *p*-nitrophenol resulted in stimulation of respiration and an increase in the amount of inorganic phosphate in the medium. In the absence of added phosphate acceptors *p*-nitrophenol and also arsenate accelerated the aerobic breakdown of fructose 1,6-diphosphate. The increase in the amount of lactic acid accumulating exceeded the increase expected from the acceleration of the breakdown of fructose 1,6-diphosphate. No effect of *p*-nitrophenol was observed under anaerobic conditions. The addition of pyruvate plus fluoride (0.005 to 0.02 M) resulted in increased aerobic formation of lactic acid. In their absence, *p*-nitrophenol produced little or no further inhibition of the Pasteur reaction and its stimulating effect on respiration was suppressed.

R. Jenness

375. Neuramin-lactose, a new compound isolated from the mammary gland of rats. R. E. TRUCCO and R. CAPUTTO, Inst. Invest. Bioq., Fund. Camp., Buenos Aires, Argentina. J. Biol. Chem., **206**: 901, 1954.

The procedure for the identification and isolation of a new lactose derivative from rat mammary gland is described. Only two components were detected, namely neuraminic (or sialic) acid and lactose, and was hence named neuramin-lactose. B. L. Larson

376. Amino acid composition of human milk. P. SOUPART, S. MOORE, and E. J. BIGWOOD, Univ. of Brussels, Belgium. J. Biol. Chem., **206**: 699. 1954.

The complete amino acid analysis of pooled human milk was accomplished by the chromatographic column procedures of Stein and Moore. Since the amino acid composition of human milk represents a standard of reference for human nutrition, it was desired to provide as complete a tabulation of the amino acids present as was possible. B. L. Larson

377. Fetuin: The mucoprotein of fetal calf serum. H. F. DEUTSCH, Univ. of Wis., Madison. J. Biol. Chem., **208**: 669. 1954.

Fetuin was further purified and characterized. It was found to be a mucoprotein with somewhat greater lability than mucoprotein iso-

lated from other sources. Aggregation occurred near pH 3 which gave variable results for the physical measurements. The monomer unit in neutral solution appeared to have a molecular weight near 45,000. B. L. Larson

378. The proteins of bovine seminal plasma. I. B. L. Larson and G. W. Salisbury, Univ. of Ill., Urbana. J. Biol. Chem., **206**: 741. 1954.

Characterization of the bovine seminal plasma proteins indicated that approximately 90% of the total nitrogen present was protein in nature and non-dialyzable. Seminal plasma proteins after exhaustive dialysis gave an extremely low test for carbohydrate and fat and contained 14.4% nitrogen. Electrophoretic studies indicated the presence of at least eleven components. Three of these accounted for the majority of the proteins present. A great similarity was evident in the electrophoretic patterns of the seminal plasma proteins obtained from bulls of various breeds and ages.

B. L. Larson

379. The proteins of bovine seminal plasma. II. Ultracentrifugal and immunological studies and comparison with blood and with serum. B. L. Larson, R. S. Gray, and G. W. Salisbury, Univ. of Ill., Urbana. J. Biol. Chem., **211**: 43. 1954.

Ultracentrifugal analyses of the bovine seminal plasma proteins revealed at least five components with the majority of the proteins in three components having $S_{20,w}$ values of about 3, 5, and 7. A great similarity was shown in the ultracentrifugal patterns of the seminal plasma proteins obtained from several bulls. Immunological analyses indicated that the seminal plasma proteins are highly antigenic. While electrophoretic and ultracentrifugal analyses indicated a closer similarity of the proteins of seminal plasma to milk serum than to blood serum, chemical and immunological comparisons suggest that the major proteins of seminal plasma are distinct entities which are either absent or are only minor constituents of blood and milk serum. B. L. Larson

380. Differential fractionation of hydrogen isotopes in liver and mammary gland. R. F. Glascock and W. G. Duncombe, Natl. Inst. for Research in Dairying, Reading, England. Biochem. J., **58**, 3: 440. 1954.

A theory is presented that predicts that biological fractionation of hydrogen isotopes during the reversible incorporation of hydrogen into any metabolite will be observed only under conditions of net synthesis and not under conditions of turnover. Since there is reason to believe that in the fatty acid metabolism of mammary gland and liver, the net synthesis and turnover are respectively the predominant processes, the incorporation of hydrogen from water doubly-labeled with tritium and deuterium into the fatty acids of these and other organs has been studied. Rats were maintained on the regime of doubly-labeled water for 11-14 days and the isotopic abundances determined in blood water and the fatty acids extracted from mammary gland, liver, perinepheric, and pup carcass fat. Defining the tritium, deterium ratio in the drinking water as unity, it was found to be unity in the blood water but significantly less than unity in all other samples assayed with the exception of the perinepheric fat in which the isotopic incorporation was too small for the isotopic ratio to be calculated accurately. The mean values for the ratios for fatty acids of maternal mammary gland and liver were 0.775 and 0.87, respectively, which are significantly different from each other. These findings are in accord with the belief that more net synthesis of fatty acid synthesis occurs in mammary gland than in liver and with the theory that under such conditions differential fractionation of hydrogen isotopes will be observed. The isotopic abundances in pup carcass fat varied considerably but the isotopic ratio was approximately the same as fatty acids of the glands from which they had been feeding.

R. Jenness

381. The lipoprotein particles in cow's milk. R. K. Morton, Dept. of Biochem., Univ. of Cambridge. Biochem. J., **57**, 2: 231. 1954.

The lipoprotein complex of cow's milk was prepared by centrifuging washed cream buttermilk at 14,000 G. and at 2° C. The casein complex of cow's milk was similarly prepared by centrifuging skimmilk. Electron micrographs of these two materials show that both occur in milk as discrete particles ranging in size from a maximum of about 200 mμ to less than 30 mμ. The lipoprotein preparation was brown in color, contained about 22% total lipid (largely phospholipid) and also contained nucleic acid and a hemochromogen, probably cytochrome c. It had the following enzymatic activities: alkaline phosphatase, xanthine oxidase, diaphorase, and DPN-cytochrome c reductase. It did not have any activity for succinic dehydrogenase and cytochrome oxidase. The lipoprotein particles of milk have properties essentially similar to those of microsomes from lactating mammary gland and other animal tissues. The name "milk microsomes" is therefore suggested for them. It is suggested that they are released into milk from the mammary gland during the normal secretory process and occur partly in association with the fat globules because of the marked tendency of the latter to adsorb them.

R. Jenness

382. Relative utilization of glucose and acetate carbon for lipogenesis by mammary-gland slices, studied with tritium, C^{13} and C^{14}. J. H. Balmain, S. J. Folley, and R. F. Glascock, Natl. Inst. for Research in Dairying, Univ. of Reading. Biochem. J., **56**, 2: 234. 1954.

This study was undertaken to determine the

relative values of acetate and glucose as sources of carbon for fatty acid synthesis by mammary tissue when both substrates are present in the incubation medium. A triple-labeling technique was used in which the methyl carbon of acetate was labeled with tritium, carboxyl with C^{13} and all the carbon atoms of glucose with C^{14}. Mammary gland slices from lactating rats incorporate into the fatty acids about eight times more glucose carbon and about one-sixth as much of acetate carbon as do slices from lactating ewes. The rat mammary tissue utilized carbon from acetate or glucose for fatty acid synthesis at approximately equal rates while that from the ewe utilized in a given time about 30 times more acetate carbon than glucose carbon. In the presence of acetate plus glucose rat mammary slices utilize less glucose carbon for fatty acid synthesis than in the presence of glucose alone. Acetate, however, has no effect on the trivial utilization for fatty acid synthesis of glucose carbon by slices of sheep udder. The addition of insulin to the incubation mixture increased the incorporation of glucose and acetate carbon into the fatty acid of rat mammary slices to about the same extent but glycerol increased the utilization of acetate carbon significantly more than that of glucose carbon. Both rat and sheep mammary tissue utilized both carbon atoms of acetate for the formation of fatty acids to an equal extent. There is no evidence that the two carbon chain is split in the course of lipogenesis. About two-thirds of the hydrogen atoms of acetate are lost or exchanged during the elongation of the chain.

R. Jenness

383. Biosynthesis of fatty acids by slices and cell-free suspensions of mammary gland. G. Popják and A. Tietz, Natl. Inst. for Med. Research, Mill Hill, London. Biochem. J., **56**, 1: 46. 1954.

The biosynthesis of fatty acids from acetate in tissue slice and cell-free suspensions of rat and sheep mammary glands was studied. In the tissue slice work pyruvate and members of the citric acid cycle stimulated the synthesis of fatty acids from acetate. These substances were particularly effective with slices of sheep udder. It was demonstrated that cell-free suspensions of the mammary gland of lactating rats and sheep synthesized fatty acids from carboxy-C^{14} acetate. In the suspensions from rat mammary gland three conditions were necessary for fatty acid synthesis: (a) aerobic incubation, (b) the oxidation of either pyruvate oxaloacetate or α-oxoglutarate and (c) the addition of ATP to some extent. The best results were obtained in the presence of oxaloacetate and ATP and when the gas phase was air instead of pure oxygen. It was shown chromatographically that the rat mammary gland suspensions synthesized all of the saturated η-fatty acids containing an even number of carbon atoms from 6 to 18 and also oleic acid.

Suspensions of sheep udder used acetate for fatty acid synthesis even without further addition although oxaloacetate had a stimulating effect in this preparation also, but ATP in a concentration of 0.01 M inhibited fatty acid synthesis. A suspension of sheep mammary gland oxidized appreciable amounts of acetate but the suspension from rat mammary glands were virtually inactive in this respect.

R. Jenness

384. Some enzymic changes in the mammary gland of rats during pregnancy, lactation, and mammary involution. A. L. Greenbaum and F. C. Greenwood, Dept. of Biochem., Univ. Coll., London, W.C. 1. Biochem. J., **56**, 4: 625. 1954.

Changes in the activity of glutamic dehydrogenase, glutamic-aspartic transaminase, cathepsin and β-glucuronidase have been studied in the mammary gland of rats at various stages during pregnancy, lactation, and mammary involution. The activities of glutamic dehydrogenase, glutamic-aspartic transaminase increased only slightly during pregnancy but rose sharply after parturition to a high level which was maintained throughout lactation and fell sharply at weaning. β-Glucuronidase activity increased sharply between the 15th and 20th days of pregnancy but then fell progressively over the period of lactation. It increased sharply after weaning during the period of mammary involution. The catheptic activity of the mammary gland increased progressively from the 10th day of pregnancy up to the 20th day of lactation and increased even further during mammary involution.

R. Jenness

385. Butyrate as a precursor of milk constituents in the intact dairy cow. M. Kleiber, A. L. Black, M. A. Brown, J. Luick, C. F. Baxter, and B. M. Tolbert, Univ. of Calif., Davis. J. Biol. Chem., **210**: 239. 1954.

Injection of intact normal dairy cows with sodium butyrate-1-C^{14} and -2-C^{14} revealed that about one-third of the transfer of carboxyl carbon of butyrate to lactose apparently passes through the carbonate pool and about one-fifth of the corresponding transfer to casein and less than one-tenth of that to milk fat pass via carbonate. The carbonate pool is by-passed by over 80% of the transfer of carbon atom 2 in butyrate to lactose and by over 90% of that to casein and milk fat. More of the 1-C^{14}, as well as of the 2-C^{14}, of the injected butyrate appeared in lactose and in casein than appeared in milk fat. The pattern of labeling milk constituents differs from the corresponding one after injection of C^{14}-labeled acetate, and in contrast to acetate, butyrate behaved in the lactating cow in a more glyceneogenic than lipogenic manner. The results are consistent with the participation of butyrate in the net synthesis of carbohydrate.

B. L. Larson

386. Studies on adaptive enzyme formation in mammals. I. Galactose metabolism. P. FEIGELSON and F. P. CONTE, Antioch Coll., Yellow Springs, Ohio. J. Biol. Chem., **207**: 187. 1954.

Studies were made to determine whether prolonged high galactose intake resulted in adaptive enzyme formation in adult rats. No evidence was found for such adaptive enzyme formation. B. L. Larson

387. Lactose metabolism. III. The reversible conversion of galactose-1-phosphate to glucose-1-phosphate. R. G. HANSEN and E. M. CRAINE, Univ. of Ill., Urbana. J. Biol. Chem., **208**: 293. 1954.

By the use of purified preparations from *Lactobacillus bulgaricus* a study was made of the waldenase type enzyme capable of interconverting galactose-1-phosphate to glucose-1-phosphate. A manganese requirement for the enzyme system as well as a pH optimum near 5.0 was established. The equilibrium of the reaction was estimated at about 21 to 27% of the galactose ester and 73 to 79% of the glucose ester. B. L. Larson

388. Studies on metalloflavoproteins. I. Xanthine oxidase, a molybdoflavoprotein. B. MARKLER, H. R. MOHLER, and D. E. GREEN, Univ. of Wis., Madison. J. Biol. Chem., **210**: 149. 1954.

Xanthine oxidase requires molybdenum for activity with cytochrome-c, but not with dyes and oxygen, thus conforming to the patterns set by other metalloflavoproteins. The activity is dependent on the presence of inorganic phosphate and is competitively inhibited by pyrophosphate. Enzymatic activity with DPNH resides in the same protein with which xanthine oxidase activity is associated, but may occur at a site different from that involved with other substrates, such as aldehyde or purines. B. L. Larson

389. A rapid, simple method for the determination of reduced, dehydro—, and total ascorbic acid in biological material. R. R. SCHAFFERT and G. R. KINGSLEY, Univ. of Calif., Los Angeles. J. Biol. Chem., **212**: 59. 1955.

A method for the determination of ascorbic acid in biological materials was developed further using 2,4-dinitrophenylhydrazine. The incubation time was reduced to 5 to 10 minutes and good accuracy is claimed comparing the test with other methods using 2,4-dinitrophenylhydrazine. B. L. Larson

390. Test confirmed. M. LOEWENSTEIN, Okla. Agr. Expt. Sta., Stillwater. Milk Plant Monthly, **44**, 3: 25. 1955.

The reliability of the Cenco test for total solids (moisture) content of milk has been further proved by additional tests that modified the original procedures. A total of 686 samples were tested, using both the Mojonnier method and the Cenco tests. The mean value of the Mojonnier tests was 12.31% total solids—that for the Cenco tests was 12.20% total solids. The average difference between methods for an individual sample was 0.244%. Duplicate Mojonnier determinations made on 85 samples showed an average difference between tests of 0.226%. Duplicate Cenco determinations on 136 samples showed an average difference between tests of 0.206%. The Mojonnier test gave results averaging slightly higher in total solids than did the Cenco test. The frequency of difference between duplicates for both methods is shown in table form. C. J. Babcock

DAIRY ENGINEERING

391. Pasteurizing apparatus of the plate type. N. R. G. EKWALL. U. S. Patent 2,705,-617. 1 claim. Apr. 5, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 1: 83. 1955.

To facilitate the rate of heating or cooling, the flow of liquids between the plates of this pasteurizer is directed by means of gaskets over baffles, molded perpendicular to the direction of flow, directly in the plates.

DAIRY PLANT MANAGEMENT AND ECONOMICS

392. Vending in schools. R. CECH, Chicago Pure Milk Assoc. Milk Plant Monthly, **44**, 4: 16. 1955.

Vending machines may be the answer to overcoming the indifference, apathy, and even hostility that exists toward the school milk program. There are 161,500 schools in the United States. Only 20% are interested enough, even with government assistance, to participate in the milk program. To encourage more participation in these programs through the use of coin-operated vending machines, it is necessary to: (1) Get a mobile, fully mechanical machine in more than one standard price and size range, vending either bottles or cartons and all in ready-to-vend position; or a mobile, semi-mechanical machine at the present medium-price range but which is protected from the curious individual who likes to punch holes in the top of the carton or bottles, insert a straw and take his fill free of charge; or the present semi-mechanical machine that does not have an exposed coin box much to the delight of the amateur safe cracker, (2) Get people to understand that milk vending machines are not something one buys and gives away to schools just to see the milk consumption increase, (3) Get school administrators and teachers to understand that in the operation of fully mechanical equipment something is bound to go wrong sometime, and (4) Get school administrators and teachers to understand that a milk vending machine will not sell much milk if students do not have access to it all the time.

C. J. Babcock

393. The cost of packaging in paper and glass. F. B. Solzan, E. B. McClain Company, Memphis, Tenn. Milk Plant Monthly, **44**, 2: 14. 1955.

Data are presented showing (1) Packaging costs according to glass and paper ratios; (2) Cost of packaging according to ratios of container sizes; and (3) Selling and delivery cost per sales point according to glass and paper ratio. Based on 60 plants handling 261,729,245 pounds of milk during April, May, and June, 1954, paper packing costs 1¢ more, paper selling and delivery cost 1 1/5¢ less. Ten of the plants packaged 100% paper; six plants packaged 100% glass; and 44 were dual operations. C. J. Babcock

394. Multiple quarts increase sales. R. W. Bartlett, Univ. of Ill., Urbana. Milk Plant Monthly, **44**, 2: 17. 1955.

Selling milk in ½-gal. and gal. containers at lower prices caused consumers to buy more. The per capita sales of milk in the Chicago market and in eight downstate markets increased from 1945 to 1950 while those for the country as a whole were declining. Between 1945 and 1950 the use of ½-gal. containers nearly doubled in the Chicago market while in downstate markets it had a wide acceptance. In each of these markets the use of the large-sized containers was directly associated with a lower unit price than that paid for single qts. It is probable that the use of the ½-gal. container was one factor causing increased per capita sales. The failure to encourage quantity sales can be attributed primarily to: (1) Laws or regulations prohibiting the use of large size containers; and (2) Groups of distributors who work in individual markets to prevent the sale of low-priced milk in quantity lots. C. J. Babcock

395. Paper goes retail. C. O. Davis, Jr., Milk Plant Monthly, **44**, 2: 21. 1955.

The experience of Producer's Dairy in Danville, Illinois, tallies closely with that of other plants using 100% paper for packaging milk and indicates that: (1) Housewives will accept paper containers; (2) Routemen like paper containers for they are lighter to handle and there are no empties; (3) Theoretically, truck payloads can be larger; and (4) Paper containers are safe and sanitary. C. J. Babcock

396. Glass saves money. C. O. Davis, Jr., Milk Plant Monthly, **44**, 2: 29. 1955.

Cost studies of the Meyer Sanitary Milk Company of Kansas City, Kan., always show that packaging milk in paper costs about a cent a qt. more than packaging it in glass. This is too much for the retail profit margin and accounts for the fact that most plants are bottling glass for retail and paper for wholesale. C. J. Babcock

397. Does price sell multiple quart? F. Nonnamaker, Associated Milk Dealers, Inc., Chicago. Milk Plant Monthly, **44**, 2: 47. 1955.

Data are presented showing that the Chicago market with a great variety of container sizes is steadily going to the sale of milk at stores in multiple quarts at prices below retail. C. J. Babcock

398. Reducing fatigue in the dairy plant. R. J. Carson. Milk Dealer, **44**, 5: 147. 1955.

There are seven ways to eliminate fatigue and increase efficiency in the dairy plant; they are: (1) have proper lighting, (2) regulate temperature and ventilation, (3) eliminate noise, (4) get plenty of nourishment, (5) avoid over-work, (6) give "relief periods" during the work day; and (7) do the job the easiest and most direct way possible. C. J. Babcock

399. Selling for profit. W. M. Roberts, N. C. State Coll., Raleigh. Milk Dealer, **44**, 5: 159. 1955.

Business people should never apologize for making a profit because without it, they will not be able to (1) employ competent personnel at competitive wages; (2) provide modern facilities for processing high quality dairy products; (3) earn a return that will attract the necessary capital; and (4) provide incentives to attract and reward competent management. Data are presented showing: (1) fixed and variable costs for packaging ½ gal. of milk; (2) fixed and variable costs for delivery of milk; and (3) gross margin as a percent of selling price of some dairy products in N. C. C. J. Babcock

400. Pros and cons of selling ice cream from retail milk routes. F. Martin, Martin-Witchwood Ice Cream Co., Inc., Springhouse, Penn. Ice Cream Rev., **38**, 9: 58. 1955.

Carrying ice cream on retail milk routes has not increased the average total sales per route, it has not gained new customers, and it has increased delivery time. Selling ice cream on milk routes in competition with their own dealers is not to be recommended for a dairy company which has a well established wholesale ice cream business.

If additional ice cream plant capacity is available, the sale of ice cream on retail milk routes will increase the ice cream sales which will make for more efficient operation of the ice cream plant. Some dairy companies located in smaller communities where the potential expansion of milk sales is limited may find it advantageous to carry ice cream on the retail routes.

Costs exclusive of the drivers' commission and truck expense were found to average $3.83 per route per week. Of this amount, $2.07 was allocated for depreciation, electricity and labor for −20° F. room and preparing route orders, $1.35 was allocated for depreciation, service,

and electricity for refrigerated units on the trucks, whereas the remaining $0.41 was the cost of additional office expense because of ice cream being carried on the milk routes.

W. J. Caulfield

401. Dairy surpluses can be disposed. G. O. TIFFANY, DISI, Washington, D. C. The Milk Prod. J., **46**, 4: 26. 1955.

DISI has sponsored a World Milk Pool plan, a world-wide industry spearheaded movement to dispose of dairy surpluses by trade and barter. This plan calls for the development of long-range markets and long-range nutritional programs in many countries throughout the world.

A Dairy Export Advisory Committee, whose membership is representative of the broad interests of the entire dairy industry, has been created by Secretary Benson. This committee has been charged with the duty of guiding the department's Foreign Agricultural Service in the administration of the "Agricultural Trade Development and Assistance Act of 1954."

The Dairy Export Advisory Committee is presently formulating recommendations to the Secretary of Agriculture which, in broad scope, contemplates the bringing together of private industry, the governments of the dairy countries and the United Nations into a "World Milk Pool" program.

Recommendations call for a long-range program of orderly exports and suggest the disposal take place through experienced dairy products distributors having normal sales operations in the overseas marketing areas.

J. J. Janzen

402. What price volume? ANON. Ice Cream Trade J., **51**, 3: 24. 1955.

The current wars of lowered prices of ice cream are resulting in lower profits for ice cream manufacturers. Assuming that most basic costs have not changed, and that a gallon of ice cream costs $1.50 and sells for $1.80, 100 units would produce $30 profit; a 5% cut in selling price would require 143 units to produce the same profit and a 15% cut would require 1,000 units to produce $30 profit. If the base price is $1, instead of $1.50, and the selling price is $1.33, it would require 125 and 250 units to produce the same profit derived from the sale of 100 units if a 5 and 15% reduction in price, respectively, were made.

W. H. Martin

403. How to plan the layout of the outlet. J. J. SHEURING, Univ. of Ga., Athens. Ice Cream Trade J., **51**, 3: 48. 1955.

There are three basic types of soft-serve stores, any of which can be modified to meet particular and specific needs: (1) the "walk-in" type of store which requires the customer to enter the store to be served; (2) the "open" type of store which permits the customer to be served without entering the interior of the building; and, (3) a combination type of store which provides facilities for customers inside the store as well as serving windows for those who prefer to remain outside.

The design should include careful planning for the most efficient use of labor and regulated traffic control of customers. Special care should be taken in planning for future expansion that will not seriously alter the basic design of the building.

Plans and specifications and building hints are given. W. H. Martin

FEEDS AND FEEDING

404. Copper complexes in grassland herbage. C. F. MILLS, Dept. of Physical Sci., Wye College, Ashford, Kent. Biochem. J., **57**, 4: 603. 1954.

Since copper deficiency syndromes in cattle and sheep frequently occur in areas where copper intake appears to be adequate, this study was undertaken to determine the status of copper in grassland herbage. The greater part of the copper in herbage was found to exist in a bound form and was extractable by organic solvents or by dilute aqueous solutions or of organic chelating agents. Evidence is presented that a slightly greater portion of the copper is present in complex form in herbage from pastures upon which sheep develop a condition of neonatal ataxia (Swayback) than in herbage from normal pastures. However, these data do not permit a conclusion as to whether or not lack of availability of copper in the herbage is responsible for the development of the copper deficient syndrome. R. Jenness

405. Sorghums and soybeans as silage crops for milk production. W. B. NEVENS and K. A. KENDALL, Ill. Agr. Expt. Sta., Urbana. Bull. 578. 1954.

Field trials were conducted from 1943 through 1947 to determine yields and suitability for silage of a number of types of sorghum, including sorgo, kafir, milo, broomcorn, and Sudan grass, and also soybeans of the grain type. Once corn hybrid was included in the tests. These crops were studied for several different factors, with yields and feeding values being given considerable attention. Much greater yields were realized from sorgos, kafirs, broomcorn, and corn than from milos and soybeans. The average yield in Ill. for the outstanding crops mentioned is 2.5 to 3 tons, whereas, under the conditions of this experiment, the yield was from 3 to 7 tons. Analyses of the different crops for protein and milk production during feeding trials were used as means of evaluating the feeding value. The yield of protein for most of the crops was less than 600 lb/acre. The protein content of sorgo and kafir was similar to that of corn. During the production trials, cows fed corn silage produced 8% more milk and declined less rapidly in milk yield than cows fed sorghum silage. R. W. Hunt

406. A method for estimating the money value of corn silage. W. B. Nevens, K. E. Harshbarger, R. W. Touchberry, and G. H. Dungan, Ill. Agr. Expt. Sta., Urbana. Bull. 576. 1954.

Five corn hybrids were grown for silage from 1947 through 1951 on highly productive land. The hybrids were known to be good producers. Beginning when the kernels were in early milk stage and continuing at weekly intervals until optimum or advanced silage stage has been reached, samples of the standing forage were obtained by field sampling. The samples were separated into ear and leaf-stalk portions, and dry matter determinations were made on each portion. Fresh forage yields were from 10 to 20 tons and dry matter yields were 3 to 5 tons per acre. The yields of ears ranged from 7 bu. at early stage to 100 at advanced silage-harvest stage. This increase in ears during the latter portion of the growing season raises a serious doubt concerning estimates of the amount of ears in silage as determined by the harvest of ripened ears from representative rows left standing in the field. Data collected during these trials show that such estimates are apt to over-rate the proportion of ears in the silage. R. W. Hunt

407. A simple milk-replacer formula for dairy calves. R. E. Leighton and I. W. Rupel, Texas Agr. Expt. Sta., College Station. Prog. Rpt. 1710. 1954.

The milk-replacer used during this experiment was a gruel prepared from the following mixture of dry ingredients: 537.5 lb. of nonfat dried milk solids, 450 lb. of dried whey, 10 lb. of Aurofac 2A, and 2.5 lb. of Quadrex "10" (stabilized vitamin A & D supplement). Cost of ingredients was $11.40. Should government nonfat milk powder be available, an appreciable reduction in the price of this milk-replacer could be realized. R. W. Hunt

408. Self-feeding storage structure. E. G. Dueringer (assignor to A. O. Smith Corp.). U. S. Patent 2,704,995. 5 claims. Mar. 29, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 5: 573. 1955.

Silage is mechanically removed from a silo by means of a worm or auger which extrudes the silage in the form of a compressed plug. The discharged plug is forced upward through the bottom of a feeding trough which rotates around the silo, thus providing feeding area for a considerable number of cows.

R. Whitaker

GENETICS AND BREEDING

409. Testing fertility in bulls. J. N. Cummings, Minn. Agr. Expt. Sta., Minneapolis. Tech. Bull. 212. 1954.

A total of 598 samples of semen from 56 bulls owned and used for artificial breeding by one of Minn. artificial breeding associations was analyzed by a series of six tests. This same semen was used for 35,545 first services. The laboratory tests consisted of: (1) rate of impedance changes in fresh undiluted semen, (2) sperm concentration, (3) ratings of motility after 24 hr. under refrigeration, (4) content of live and dead sperm after 24 hr. refrigerated storage, (5) respiration rate after 24 hr. refrigerated storage, and (6) content of morphologically abnormal spermatozoa in fresh samples. The degree of fertility in bulls was most accurately determined from a combination of five of the semen tests employed, and this combination is called the fertility index. As to the value of single tests, those based on motility of spermatozoa measured the degree of fertility most accurately. Phase microscopy offered many advantages over the use of ordinary bright field microscopy. Sperm concentration and content of abnormal sperm had little value on predicting fertility. An inter-relationship existed among rate of impedance changes, motility, respiration rate, and content of live sperm. R. W. Hunt

ICE CREAM

410. Corn syrup in an ice cream plant. L. G. Drusendahl, The Hubinger Co., Keokuk, Iowa. Ice Cream Rev., **38**, 9: 59. 1955.

The composition and properties of the different types of corn syrup are presented, as well as typical systems of handling liquid corn syrups in the ice cream plant.

W. J. Caulfield

411. HTST pasteurizing of ice cream mixes with the Roswell heater. R. O. Tardiff, Breyer Ice Cream Co., Philadelphia, Pa. Ice Cream Trade J., **51**, 3: 58. 1955.

The flow diagram of the hook-up in the Newark plant is presented and a description of the process is given. The unit tested had a capacity of 12,000 lb. of mix per hour.

All the controls are mounted within the instrument panels above the cabinet holding the pasteurizing tubes. The equipment requires comparatively little floor space and is neat in appearance. W. H. Martin

412. The significance of coliforms in ice cream. J. M. Frayer, Vt. Agr. Expt. Sta., Burlington. Ice Cream Trade J., **51**, 3: 28. 1955.

The coliform test, phosphatase, and total plate count make a valuable team when properly used and evaluated in the sanitary control of ice cream. When used as a line test, the coliform count helps to determine the point or points at which cleaning or germicidal treatment and protection are weak. It shows whether or not materials added at the freezer have been adequately protected. W. H. Martin

413. Machine for making coated frozen confection balls. R. N. REINHARD (assignor to Foremost Dairies, Inc.). U. S. Patent 2,705,462. 5 claims. Apr. 5, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 1: 44. 1955.

A machine is described for making frozen confections in the shape of spheres. Two wheels, having semispherically shaped depressions in the periphery, rotate in such a manner that the circumferences touch and the two half molds come together to form a complete mold. A mold filling means is provided. The frozen molded pieces are ejected from the molds by distorting the resilient walls of the cavities.
R. Whitaker

414. Apparatus for making frozen confections. C. K. NELSON (assignor to Eskimo Pie Corp.). U. S. Patent 2,704,442. 6 claims. Mar. 22, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 4: 422. 1955.

An arrangement of tubes carrying refrigerant for cooling molds for making frozen novelties. R. Whitaker

MILK AND CREAM

415. Rancidity development in cream from cold-separated raw milk. G. M. TROUT, J. M. JENSEN, and E. S. HUMBERT, Mich. Agr. Expt. Sta., East Lansing. Quart. Bull., **37**, 3: 392. 1955.

Lipolysis is affected by temperature changes, nature, and extent of agitation, as well as feed. Winter feeding practices and associated temperatures are considered as uncontrollable factors. Movement of milk in pipelines, mechanical agitation for initial cooling, agitation before extracting fat sample, pumping to transport truck, then into storage tanks, and finally through a clarifier and/or cold separator, all contribute a considerable amount of agitation. The exact cause for lipolysis in cold separated cream used in these experiments was not determined. However, the results indicated that fresh raw cream regardless of season was not rancid. Pronounced rancidity was detected in Feb.-produced cream stored 4 days but not in June-produced cream. R. W. Hunt

416. Precision calipers for greater accuracy in reading Babcock tests. ANON. The Milk Prod. J., **46**, 4: 38. 1955.

A new mechanical caliper which provides easier and more accurate reading of Babcock tests has been developed. This particular instrument consists of pointers with hairline accuracy and micrometer adjustment, magnification, and good illumination. Pointers on this improved instrument are similar to the hairline in a surveyor's telescope, and are regulated with rack and pinion for close adjustment. The operator looks into a brilliantly-lighted background through a magnifying lens. Reading tests with a precision instrument will tend to minimize the possibilities of inaccurate readings. J. J. Janzen

417. Low fat and nonfat dairy products. PAUL J. FINDLEN, N. Y. State Joint Legislative Committee on Imitation Milk Products and Problems. Milk Dealer, **44**, 5: 108. 1955.

With the growing trend toward drinking low-fat or skimmilk, using lighter cream, eating more cottage cheese, and enjoying more low fat frozen dairy desserts, it is timely to consider amendments to existing state laws now prohibiting such products that would be in the interests of dairymen as well as consumers.
C. J. Babcock

418. Maintain quality on every-other-day delivery. S. J. WEESE and J. E. FIKE, W. Va. Univ. Milk Plant Monthly, **44**, 4: 13. 1955.

Delivery every other day or three times a week is sufficient for pasteurized or pasteurized-homogenized milk of good quality, providing the processor and consumer use reasonable care in the handling of the milk. A three-months study with 18 housewives showed that: (1) Prompt refrigeration on delivery makes a significant difference in the flavor and keeping quality, as well as vitamin values, (2) Prompt return to the refrigeration and tight capping or closing after part of the milk has been used protects the fresh flavor. Milk which stands unrefrigerated and uncovered for only half an hour loses some quality and freshness, and (3) Good housekeeping in the refrigerator pays off in the fresher milk. C. J. Babcock

419. Dispenser closure having tilt valve and aligning cap. J. W. SOFFER (assignor to Development Research, Inc.). U. S. Patent 2,704,621. 5 claims. Mar. 22, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 4: 469. 1955.

A valve and protecting cap are described for releasing cream or other similar product from a gas pressurized can. R. Whitaker

420. Restricted-tilt dispenser valve having flanged stem. J. W. SOFFER (assignor to Development Research, Inc.). U. S. Patent 2,704,622. 4 claims. Mar. 22, 1955. Offic. Gaz. U. S. Pat. Office, **692**, 4: 469. 1955.

A description is given of a valve for dispensing whipped cream or similar products from a can packed under gas pressure. The valve opens and discharges the product when the dispensing outlet is tilted slightly to one side by the finger. R. Whitaker

PHYSIOLOGY AND ENDOCRINOLOGY

421. Dietary carotene and the degree of esterification of vitamin A in the milk and blood of cows. R. CHANDA, H. M. CLAPHAM, and E. C. OWEN, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **56**, 3: 453. 1954.

Four lactating Ayrshire cows were fed in alternate periods carotene-free diets and diets containing carotene. The intakes of carotene in the successive carotene feeding periods increased as the experiment progressed. In con-

firmation of previous work by the same authors, carotene deprivation caused the amount of vitamin A alcohol in the milk to increase but it did not affect the concentration of vitamin A alcohol in the blood serum. Removal of carotene from the diet caused vitamin A ester to disappear completely from the blood serum. This ester reappeared when carotene was again fed. These results, as well as earlier results with thyroxin-treated animals, suggest that increases in vitamin A alcohol in the milk of cows indicates a transfer of reserves of vitamin A from the cows' liver to the milk. R. Jenness

422. Studies on pituitary lactogenic hormone. XIV. A simplified procedure of isolation. R. D. Cole and C. L. Li, Univ. of Calif., Berkeley. J. Biol. Chem., **213**: 197. 1955.

From 1 kg. of whole sheep pituitary glands, about 2 g. of lactogenic hormone (prolactin) was isolated. The preparation was essentially free of growth hormone, ACTH, thyrotropin, the gonadotropins, and intermedin but had crop sac-stimulating activity of 35 i.u. per mg. and was also potent with respect to luteotropic action. Physiochemical investigations indicated that the hormone behaves as a homogeneous protein. B. L. Larson

423. Relative galactopoietic effects of 3:5:3′-triiodo-l-thyronine and l-thyroxine in lactating cows. S. Bartlett, A. W. A. Burt, S. J. Folley, and S. J. Rowland, Natl. Inst. Research in Dairying, Univ. of Reading. J. Endocrinol., **10**: 193. 1954.

The compound 3:5:3′ triiodo-l-thyronine (TIT) has been isolated from the blood and the thyroid gland and reported to have a greater biological activity than thyroxine. Forty cows were divided so that 8 were fed 75 mg. thyroxine daily for 3 weeks, 24 were fed varying doses of TIT and 8 were controls. In another experiment 4 cows were injected with 5 mg. TIT daily for 2 weeks and 4 were injected with 5 mg. thyroxine. The oral thyroxine increased the milk yield, fat percentage and decreased the alkaline phosphatase. Oral TIT had no effect except for a slight increase in milk yield. Injected thyroxine increased the yield, fat percentage, and per cent of solids not fat and a decrease in the alkaline phosphatase. Injected TIT had the same effect as injected thyroxine but the effect was greater in all cases. The authors suggest that the low oral potency of TIT as compared to thyroxine is due to its greater solubility in the rumen and that the microorganisms quickly become adapted to inactivate it. R. L. Hays

424. The effect of adrenaline on the milk-ejection response of the sow. W. G. Whittlestone, Ruakura An. Research Sta., Hamilton, N. Z. J. Endocrinol., **10**: 167. 1954.

Intravenous injection of 200 μg. of epinephrine had an inhibitory effect on the milk ejection responses to 0.5 IU of oxytocin. The inhibition was almost complete when epinephrine injections preceded oxytocin injections by 20 seconds or less. The inhibition decreased as the interval between the two injections increased but the inhibition was detectable up to an interval of 2 minutes. When the two hormones were mixed before injection, it was necessary to reduce the oxytocin to 0.2 IU before any inhibition due to 200 μg. of epinephrine was noted. The degree of milk ejection was determined by the duration of response and intramammary pressure. Epinephrine alone had no effect on intramammary pressure. R. L. Hays

425. The influence of suckling on food intake and growth of adult female rats. P. M. Cotes and B. A. Cross. J. Endocrinol., **10**: 363. 1954.

Suckling of female rats with the galactophores cut to prevent milk removal and normal lactating rats caused the females to gain significantly more weight than unsuckled controls. The increased rate of gain was paralleled by an increase in food intake even in the suckled rats with the galactophores cut. As a result the suckling caused an increase in food intake in excess of the needs for milk production. The mechanism involved does not appear to include growth hormone or prolactin. R. L. Hays

426. Direct hormone inactivation by extracts of Lithospermum ruderale. R. L. Noble, E. R. Plunkett, and R. C. B. Graham. J. Endocrinol., **10**: 212. 1954.

Cold water extracts of *Lithospermum ruderale* almost completely inactivated pregnant mare serum, pituitary gonadotrophin, prolactin, and thyrotrophin upon incubation for 2 hr. at 37° C. when they were tested by gonad weights in intact immature and hypophysectomized male and female rats, thyroid weights of male guinea pigs and crop sac weights of pigeons. *L. ruderale* extracts partially inhibited the stimulatory effect of chorionic gonadotrophin on the gonads of intact and hypophysectomized male and female rats but had no apparent effect on the normal action of growth hormone, adrenocorticotrophin vasopressin or insulin. In the presence of *L. ruderale*, gonadotrophins caused a greater stimulation of the interstitial cells of the ovary and uterine weight than of the weight of the ovaries indicating that the inhibition was acting primarily through its effect on the luteinizing hormone. Thyrotrophin was selectively inactivated in a mixture of thyrotrophin and growth hormone. R. L. Hays

427. Further observations on oocyte numbers in mature rhesus monkeys (Macaca mulatta). S. H. Green and S. Zuckerman, Univ. of Birmingham, England. J. Endocrinol., **10**: 284. 1954.

The numbers of oocytes in the various stages

of development were determined in the serial sectioned ovaries of 42 monkeys. There were no differences in the right and left ovaries or in the early, middle, or last thirds of the cycle. A log plot of the number of oocytes against body gave a straight line with a significant regression ($b = -1.81 \pm 0.422$). Since the older monkeys are usually the heavier, this is evidence that the number of oocytes declines with age.
R. L. Hays

428. The effect of adrenocorticotrophic hormone on the yield and composition of the milk of the cow. D. S. Flux, S. J. Folley, and S. J. Rowland, Natl. Inst. Research in Dairying, Univ. of Reading. J. Endocrinol., **10**: 333. 1954.

Injection of 100 or 200 IU of ACTH in single doses or 100 IU every second day for 10 days into 15 cows in the declining phase of lactation caused a significant drop in milk production which returned to near normal after cessation of injections. The ACTH caused an increase in the percentage of fat and protein and a small but not significant decrease in lactose. The decline in milk yield was correlated with decrease of eosinophils in the blood. There was no effect on the sodium and potassium levels in the milk.
R. L. Hays

SANITATION AND CLEANSING

429. Lye storage indicated for rubber parts. T. J. Claydon, Dept. of Dairy Hus., Kan. State Coll., Manhattan. Milk Plant Monthly, **44**, 4: 26. 1955.

A detailed study of relative merits of wet and dry storage for rubber parts of milking machines showed that with new liners, under laboratory conditions of contamination and treatment, there was little practical difference between the effectiveness of lye storage and dry storage. With used liners, on the other hand, differences often were considerable, with dry stored liners having a log average bacterial count 20 times as great as that for lye stored liners. With milk tubes, under practical service conditions lye storage treatment resulted in approximately the same bacterial counts in the used tubes as in the new tubes. However, with dry storage, the used tubes had much higher counts than new tubes. The degree of contamination in both was considerably greater than with lye storage. When the efficacy of lye rack storage for the used liners was compared with dry storage under practical operating conditions, lye storage again proved to be the most effective.
C. J. Babcock

430. Can washing machine. W. G. Schulze (assignor to Damrow Brothers Co.). U. S. Patent 2,705,965. 1 claim. Apr. 12, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 2: 182. 1955.

A straight-line milk can and lid washer is described, which handles cans of various neck diameters. The valves which admit cleaning and rinsing fluids to the can sprays are hydraulically operated when a can is positioned correctly for spraying.
R. Whitaker

431. Power washing cream separator with back type metering valve. J. R. Orelind and W. H. Harstick (assignors to International Harvester Co.). U. S. Patent 2,705,970. 10 claims. Apr. 12, 1955. Offic. Gaz. U. S. Pat. Office, **693**, 2: 184. 1955.

A cleaning solution is metered into a rotating separator bowl by means of a valve located in the bottom of the supply tank and activated by a float which rises and falls with the liquid level in the supply tank.
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